Environmental Pollution 208 (2016) 532–536

Contents lists available at ScienceDirect

Environmental Pollution

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

Short communication

Do cytostatic drugs reach drinking water? The case of mycophenolic acid

Helena Franquet-Griell [a], Francesc Ventura [a], M.Rosa Boleda [b], Silvia Lacorte [a, *]

[a] Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18–26, 08034 Barcelona, Catalonia, Spain
[b] Aigües de Barcelona, S.A. General Batet 5–7, 08028 Barcelona, Catalonia, Spain

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form
16 October 2015
Accepted 18 October 2015

Keywords:
Mycophenolic acid
River water
Drinking water
Liquid chromatography
Tandem mass spectrometry

ABSTRACT

Mycophenolic acid (MPA) has been identified as a new river contaminant according to its wide use and high predicted concentration. The aim of this study was to monitor the impact of MPA in a drinking water treatment plant (DWTP) that collects water downstream Llobregat River (NE Spain) in a highly densified urban area. During a one week survey MPA was recurrently detected in the DWTP intake (17–56.2 ng L^{-1}). The presence of this compound in river water was associated to its widespread consumption (>2 tons in 2012 in Catalonia), high excretion rates and low degradability. The fate of MPA in waters at each treatment step of the DWTP was analyzed and complete removal was observed after pretreatment with chlorine dioxide. So far, MPA has not been described as water contaminant and its presence associated with its consumption in anticancer treatments is of relevance to highlight the importance of monitoring this compound.

1. Introduction

Cytostatic drugs have emerged as new water contaminants due to their wide use in cancer treatments (Buerge et al., 2006). In Catalonia (NE Spain), the total consumption of 132 different anticancer drugs ranged from 4.7 t to 4.9 t during the period 2010–2012 (Franquet-Griell et al., 2015). Among them, mycophenolic acid (MPA) accounted for 40% of total drugs administered, equaling 1.9 t yr^{-1} corresponding to a consumption per capita of 704 μg $inhab^{-1}$ d^{-1}. Consumption data has been used recently to calculate the predicted environmental concentration in rivers (PEC_{river}), which is a value that gives an estimate of the level of exposure for a given scenario and is thus essential for an initial indication of environmental impact (Kelly et al., 2003). According to a previous study (Franquet-Griell et al., 2015), among 132 cytostatic compounds, MPA was expected to be found in Catalonian rivers at the highest concentration (77.4 ng L^{-1}), which exceeds the threshold value for an individual drug for further environmental risk assessment (10 ng L^{-1}), according to the European Medicines Agency (EMEA) (EMEA, 2006). MPA, classified in the Anatomical Therapeutic Chemical system (ATC) as L04AA06, is used basically as renal, cardiac and hepatic allogeneic prophylactic treatment against organ rejection. After administration, 60% of the drug is excreted in the urine as mycophenolic acid glucuronide whereas 3% remains unchanged (Drugs Information Database, 2014). Once it reaches sewage water and enters a wastewater treatment plant (WWTP), the glucuronide metabolite is deconjugated and the parent compound is formed again. Its estimated removal rate is of 41% (Royal Society of Chemistry, 2014), and therefore, this compound has great chances to reach surface waters. MPA is a weak organic acid with a solubility of 22 mg L^{-1} (Royal Society of Chemistry, 2014), a predicted pKa from 3.57 to 4.61 and a partition coefficient octanol–water from 2.8 to 4.2 (depending on the database) (Chemical Book, 2008; Drug Bank Database, 2013; Royal Society of Chemistry, 2014) and this suggests that it will be preferentially detected in water. Once introduced in the water cycle it could reach tap water if the treatment is not efficient. The toxicological effects of MPA on human beings are unknown, but its mode of action is based on the non-competitive, selective and reversible inhibition of the inosinmonophosphate dehydrogenase, so it inhibits *de novo* synthesis of the nucleotide guanosin, without incorporation to DNA. It can have negative effects on the long term like cyclophosphamide, tamoxifen or melphalan, which are known to be carcinogenic (IARC, 2015). Thus, the importance of MPA monitoring in water intended for human consumption.

Whereas ultra high performance liquid chromatography

* Corresponding author.
E-mail address: slbqam@cid.csic.es (S. Lacorte).

http://dx.doi.org/10.1016/j.envpol.2015.10.026
0269-7491/

coupled to tandem mass spectrometry (UHPLC-MS/MS) methods have been developed to analyze this compound in biological samples for clinical therapeutic drug human biomonitoring (Klepacki et al., 2012), to our knowledge, no one has attempted to determine this compound as a water contaminant. Because of its potential occurrence in river waters, drinking water becomes at risk, especially when river water constitutes the main source for purification. The study site is the drinking water treatment plant (DWTP) that supplies water to the Barcelona city (2,000,000 inhabitants). This plant collects water from the Llobregat River, a river which receives the impact of 139 cities and villages, the discharges of 94 WWTPs and 571 authorized waste discharges from industries and agricultural fields (Agència Catalana de l'Aigua, 2015). The DWTP intake is settled near the mouth of the river and close to the city of Barcelona, downstream of many urban, industrial discharges and run-off and therefore, it represents a worst case scenario. The aim of this study was to determine the occurrence of MPA in a drinking plant considering water collection and all the purification treatments. For such purpose, we developed and validated an UPLC-MS/MS method to accurately quantify MPA in water.

2. Experimental

2.1. Chemicals and reagents

MPA and MPA-d_3 pure analytical standards of ≥98% purity were acquired from Sigma–Aldrich (St. Louis, USA). MPA stock standard solution was prepared at a concentration of 1000 ng μL^{-1} in methanol, and working solutions at 10 ng μL^{-1}. Methanol, acetonitrile, acetone (SupraSolv grade) and HPLC water (LiChrosolv grade) were supplied by Merck (Darmstadt, Germany). Oasis HLB 200 mg solid phase extraction cartridges (SPE) were from Waters (MA, USA).

2.2. Sampling

Water samples (1 L) were collected from a DWTP located close to the city of Barcelona (Catalonia, Spain). This plant collects raw water from the Llobregat River and is treated following the order: dioxychlorination of river raw water, coagulation, flocculation, settling, sand filtration and groundwater addition to improve water quality. The filtered water is then split in two parallel purification lines, one employing conventional treatment with ozonization and GAC (granular activated carbon) filtration (~70% of the total flow), and the other, advanced treatment with ultrafiltration and reverse osmosis (~30%). Both treated waters are then blended, chlorinated and distributed. Samples were collected during 7 consecutive days (25th to 31st January 2014) and since MPA was always detected, its evolution along the treatment lines and in finished water was determined in two further sampling campaigns performed in the following days. Samples were taken throughout the DWTP according its hydraulic retention time. The sampling campaign was performed in winter, which is the season when the highest contaminant concentrations of drugs are detected in this area (Boleda et al., 2009).

2.3. Extraction and analysis

Samples were acidified at pH 2 with HCl 37% and afterward extracted with an automated solid-phase extraction apparatus (Dionex Autotrace 280, Thermo Scientific). One hundred mL of water were spiked with 5 ng of MPA-d_3 as internal standard (IS). Oasis HLB 200 mg SPE cartridges were conditioned with 6 mL MeOH and 6 mL H_2O at 3 mL min^{-1} and then the sample was loaded at a flow rate of 1 mL min^{-1}. Cartridges were rinsed with 3 mL 100 mM NH_4OAc in H_2O, dried during 30–45 min under a current of nitrogen at 5 mL min^{-1} and eluted with 4 mL MeOH and further 4 mL HCOOH:MeOH (5:95). Samples were then evaporated to almost dryness in a TurboVap under a current of nitrogen at 25 °C and transferred to a 2 mL chromatographic vial with 1 mL of ACN as washing solvent. Finally, samples were evaporated to dryness and reconstituted to 100 μL of a 50:50 mixture (0.1% HCOOH in ACN and 0.1% HCOOH in HPLC water).

UHPLC conditions were optimized to obtain good resolution and sample throughput. An Acquity Waters, USA, system connected to a Quattro-micro triple quadrupole detector (UHPLC-MS/MS) was used to determine MPA. An Acquity UPLC BEH C18 column (100 mm × 2.1 mm ID, particle size 1.7 μm) was used at a flow rate of 0.3 mL min^{-1}. The mobile phase composition consisted of binary mixtures with 0.1% HCOOH in water (A) and 0.1% HCOOH in acetonitrile (B). Gradient elution started at 95% A and 5% B, increased to 70% B in 10 min (using a slight convex curve) and increased to 100% B in 5 min. Initial conditions were attained in 1 min and the system was stabilized for 1 min. Ten μL were injected. MPA was measured under positive electrospray ionisation (ESI+). Flow injection analysis (FIA) was performed to determine the optimum cone voltage (between 10 and 100 V) that produced the molecular ion and the optimum collision energies (between 5 and 50 eV) to obtain at least two intense fragments. Finally, acquisition was performed in selected reaction monitoring (SRM) mode using two transitions from $[M+H]^+$ precursor ion to product ions to identify each compound. The transitions used as well as the optimized cone voltages and collision energies are given in Fig. 1. Internal standard quantification was performed. The data were acquired and processed using the MassLinx v.4.1 software package.

2.4. Quality control/Quality assurance

The method was assessed for accuracy, linearity, sensitivity, selectivity, extraction efficiency and matrix effects. Intra and inter-day accuracy were determined by injecting a 0.5 ng μL^{-1} standard during 5 consecutive injections and in 5 different days. Linearity was studied over a concentration range of 0.005–1 ng μL^{-1}, with IS kept at a constant concentration of 0.4 ng μL^{-1}. Sensitivity was determined by calculating the instrumental limits of detection (IDL) and method detection limits (MDL). IDL was calculated as the amount of analyte that gives a signal to noise ratio of 3 (S/N = 3) using the standard at 0.005 ng μL^{-1}. MQL was calculated as the concentration that gave a S/N = 10 and was determined from the spiked river water at 10 ng L^{-1} and gives information on the sensitivity of the method, considering the extraction and analytical procedure. Extraction efficiencies were determined by spiking Llobregat river water and from pristine mountain creek water at a concentration of 50 ng L^{-1} and performing the analysis in triplicates. At the same concentration, the matrix effect (ME) was evaluated using the following equation:

$$ME\,\% = \left(1 - \frac{I_{river} - I_{blank}}{I_{creek}}\right) \cdot 100 \quad (1)$$

where I_{river} was the MPA peak intensity in spiked Llobregat river water, I_{blank} in unspiked Llobregat river water (as MPA was always detected) and I_{creek} in the spiked pristine creek water. Identification criteria included the retention time and two transitions, one used for quantification and the other for confirmation, and the ion ratio, as suggested by the European Union Decision 202/657/EC (August 17, 2002).