Journal of Pharmaceutical and Biomedical Analysis 129 (2016) 571–580

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Identification of markers of cancer in urban sewage through the use of a suspect screening approach

Laura Ferrando-Climent[a,b,*], Malcolm J. Reid[c], Sara Rodriguez-Mozaz[a], Damià Barceló[a,d], Kevin V. Thomas[c]

[a] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain
[b] Tracer Technology department, Oil and Gas section, Institute for Energy Technology (IFE), P.O. box 40, NO-2027 Kjeller, Norway
[c] Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, NO-0349 Oslo, Norway
[d] Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form 28 July 2016
Accepted 1 August 2016
Available online 2 August 2016

Keywords:
Suspect screening
retrospective analysis
liquid chromatography coupled to high resolution mass spectrometry
chemotherapy drugs
metabolites
biomarkers

ABSTRACT

The administration of anticancer drugs during chemotherapy treatments has increased considerably in recent years, and based on the growing incidence of cancer worldwide there is a foreseen increase in their use over the coming years. Many anticancer drugs are not removed by conventional wastewater treatment plants (WWTPs) and can therefore reach the aquatic environment and potentially threaten aquatic life. The objective of this work was to apply a *suspect screening* methodology to detect chemotherapy and radiotherapy drugs and their related compounds such metabolites and/or biomarkers in wastewater. The use of logical pre-determined criteria to refine the suspect list down to a relatively small number of relevant compounds greatly improved the efficiency of the analysis. Mass accuracy, isotopic patterns and predicted retention time were used to tentatively identify the suspects. Successful identification of cancer-related suspects included two antineoplastic hormones, two X-ray contrast agents and a pyrrolizidine alkaloid related to an herbal medicine. This is the first time that a suspect screening paradigm has been successfully applied to the identification of pharmaceuticals and biomarkers related to chemotherapy in wastewater.

1. Introduction

Growth in the incidence of cancer in the human population has led to an increase in the use of chemotherapy drugs with a further rise in the use of these drugs foreseen in the coming years [1]. As a result, greater attention needs to be paid to the occurrence of anticancer drugs in the environment and any potential ecological consequences. Although chemotherapy drugs are typically administered in hospitals, 75% of the treatments are outpatient therapies [2] and therefore cytostatic compounds can reach the aquatic environment via hospital or domestic wastewater with wastewater treatment plants (WWTPs) being the ultimate source [3,4] It is also important to take into account that cytostatic compounds, as with many other pharmaceuticals, are excreted as both parent compound and as metabolites that may have the same mode of action as the parent cytostatic compound or potentially even greater activity. This is the case for 4-hydroxytamoxifen, a metabolite of tamoxifen, which is a more potent estrogen receptor antagonist than the parent compound [5]. Likewise, specific biomarkers produced by the body whilst suffering from cancer and during oncologic treatments, such α-fenotroin (prostate cancer), inositol (hepatocellular carcinoma), normethanephrine (hepatocellular carcinoma) [6], among other compounds, are present at higher or lower levels in urine. For instance α-fenotroin is present at concentrations of up to 50 ng/mL in the serum/urine of patients with liver cancer [6]. Since these substances are concomitant with chemotherapy drugs they have the potential to provide useful information about the prevalence of different types of cancer as well as about the chemotherapy drugs consumed in the population served by a WWTP.

The high cost of chemotherapy pharmaceutical reference standards, often based on a challenging and expensive synthesis, and especially their particular hazard to health or safety, makes conventional target analysis of these compounds difficult in most of the environmental laboratories due to the special and expensive safety conditions required for their handling (analyst training for cytotoxic handling and spills, personal protective equipment, bio-

* Corresponding author.
E-mail address: laurafc@ife.no (L. Ferrando-Climent).

http://dx.doi.org/10.1016/j.jpba.2016.08.001
0731-7085/

logical safety cabinet or similar category hood, specific containers for residues, etc). Nevertheless different analytical methodologies without the use of standards have recently been developed for the screening of micropollutants in water samples as a qualitative mode of assessment. In general, these novel approaches are based on high-resolution mass spectrometry (HRMS) using time-of-flight (TOF) or Orbitrap mass spectrometers often coupled to liquid chromatography (LC) [7,8]. LC-HRMS allows the wide-scope screening of organic pollutants without the pre-selection of analytes and without using authentic reference standards [9]. Known compounds suspected of being present in environmental samples can be retrospectively screened following data acquisition [10].

In this work, a "*suspect screening*" approach was applied to wastewater samples collected from Oslo, Norway for the purposes of detecting and identifying (bio-)markers associated with prostate cancer and breast cancer.

Oslo is an ideal sampling location because Norway has a strictly controlled system for the prescription and dispensing of medications that is well-managed by the Norwegian Institute of Public Health (NIPH) through the Norwegian prescription database (NORDP) (www.nordp.no). This database provides accurate information on the use of therapeutic agents in any given region/city.

Prostate cancer and breast cancer were selected as the targets because a review of the World Health Organization databases reveals that these two cancers are the most frequently diagnosed in Norway (www.who.com).,

2. Material and Methods

2.1. Chemical reagents

LC-MS grade water and acetonitrile were supplied by Merck (Darmstadt, Germany). Reagents, such as formic acid 98% (HCOOH), were supplied by Sigma-Aldrich (HPLC-grade) and leucine encephalin and ethylenediaminetetraacetic acid disodium salt (EDTA) was supplied by Sigma-Aldrich (Germany).

2.2. Sampling campaign

The urban area selected for this study, Oslo, has a population of approximately 647,000 residents. Both VEAS and Bekkelaget WWTPs, which are located on the Oslofjord, receive the urban wastewater from the city and various surrounding municipalities. VEAS receives wastewater from different sources such as hospitals, including the region's largest hospital where most of the chemotherapy treatments are administered, and industry in addition to domestic sewage from the west of city. Bekkelaget WWTP receives wastewater from the east urban collectors including small health centres such a psychiatric clinic (Lovisenberg Diakonale Sykehus Psykiatrisk). The volume of wastewater processed by both WWTPs is estimated to be between 100 and 110 million m^3 year-^{1} for VEAS and about 42 millon m^3 $year^{-1}$ for Bekkelaget. In total, 24 samples (four-hourly time-proportional composite samples of 1 L, providing a total of six samples per day over a period of four weekdays) were collected from the wastewater influent of both WWTPs. All the samples were collected in amber glass bottles that were pre-rinsed with Milli-Q water and were analysed immediately after the sampling.

2.3. Sample pre-treatment

The analytical methodology previously developed by Ferrando-Climent et al. was adapted for sample pre-treatment [3]. Sample pre-concentration was performed using solid disk sorbents in an automated system (SP-DESK® 4790, was provided by Horizont Technologies (New Hampshire, U.S.). This system allows the use of high flow rates during sample loading allowing increased sensitivity since the volume loaded is higher than that of a conventional SPE cartridge.

EDTA was added to the sample to obtain a final concentration of 0.1% (g solute g^{-1} solution), as it is well known that it improves the extraction of certain organic compounds that may be bound to residual metals [3,11–13]. Each sample (800 mL) was loaded by the automatic system (60 mL·min^{-1}) onto a hydrophilic-lipophilic balance (HLB) medium size disk (Atlantic ® SPE disk 47 mm and 500 mg of sorbent) with a glass fibre disk pre-filter just placed onto the extraction disk (Atlantic pre-filter ® fast flow, 50 mm). Both disks were first conditioned using 6 mL of methanol (2 × 3 mL) and followed by 3 mL 0.1% formic acid solution (2 × 3 mL). Elution was performed using 15 mL (3 × 5 mL) of pure methanol. The extract was evaporated to dryness under gentle nitrogen stream and reconstituted with 1 mL of methanol-water (10:90, v/v). Samples were subsequently filtered through 0.45 μm PVFD filter (Sartorius) before injection. Note however that in order to recover any non-polar molecules that may have adsorbed to the filter, 1 mL of methanol was passed through the filter after the sample filtration. Both sample extracts, aqueous and organic, were collected for analysis.

There is a common limitation in the methods based on non-target analysis: the estimation of the limit of detection, recovery or ionization parameters are not possible to assess since the analytical methodology is based on the screening of suspected molecules and (frequently) no reference standards are available.

2.4. UPLC-QTOF Method

Analysis was performed using ultra-performance liquid chromatography system (Waters Corp. Mildford, MA, USA) equipped with a binary solvent system (Mildford, MA, USA) and a sample manager, coupled to a quadrupole-time of flight mass spectrometer (Xevo-G2 S QTOF, Waters Corp., Mildford, MA, USA). Chromatographic separation was performed using an Acquity BEH C18 column (150 mm x 2,1 mm i.d. 1,7 μm particle size; Waters Corp. Mildford, MA, USA) where the separation was performed in 15 minutes using a binary mobile phase of formic acid 0.1% (solvent A) and acetonitrile (solvent B) at 0.4 mL/min with a standardised gradient for blind screenings [14]. The gradient elution starts with 87% A and then increasing B to 95% in 15 min: Solvent A, held for 0.5 min; 0.5–10 linear rate to 50% B, 10–10.75 linear rate to 95% B, held for 0.5 min; reconditioning with a linear rate to 87% A, 12.50–15 min. 10 μL of extracted samples (both organic and aqueous extracts) were injected in the system.

The MS analysis was performed using positive electrospray ionization (ESI +) over the range of 50 − 800 Da with a scan time of 0.2 s and an inter-scan delay of 0.05 s. Two simultaneous acquisition modes with different collision energies were used for the MS experiments: (i) a low energy mode (LE) where collision energy of 4 eV was selected for detection of molecular ions [M-H]+, and (ii) a high energy (HE) mode with a collision energy ramp from 15 eV to 40 eV for detection of fragment ions.

2.5. Suspect list

The suspect list was based on two cancer types, prostate and breast since they are the most frequently diagnosed in Norway (www.who.com). The chemotherapy drugs associated with the treatment of these cancers, together with a large group of pharmaceuticals/therapies such mitotic inhibitors, anti-metabolites, hormones and immunotherapeutic agents were identified with the NORDP databases. Following the selection of specific therapies (> 1420 compounds), only the pharmaceuticals used during 2013 in Oslo for both males and females that were prescribed at sufficiently