Análisis cualitativo y cuantitativo de fármacos en peces por espectrometría de masas de alta resolución de cuadrupolo-tiempo de vuelo

Programa de Doctorado
Química Analítica y Medio Ambiente

Juan Manuel Peña Herrera

Abstract

Pharmaceuticals are, mostly synthetic, chemical substances used for the prevention, diagnosis, treatment and/or cure of diseases, in both animals and humans. The changing lifestyle that includes malnutrition, alcohol consumption, smoking, drug abuse, stress, unhealthy diet, long working sessions, sedentarism, and so on has promote the consumption of pharmaceuticals. This growth in consumption is particularly noticeable in developed countries, for example those that are part of the Organization for Economic Cooperation and Development, OECD. In some cases, the increament of pharmaceuticals compsumption has raised up to 1500 % in countries as Denmark in 15 years (e.g. for lipid modifying agents). Although the main objective of the use of pharmaceuticals is to improve the quality of life of humans and of society in general, the continuous release of drug residues into the environment through a variety of sources may result in long-term exposure of wildlife with potential undesired effects on non-target organisms.

Once pharmaceuticals have entered the target organism, in humans most frequently by the oral route, they are subject to biotransformation processes, which convert the parent drug into one or several metabolites of usually reduced activity towards the biological receptor. The intact fraction of the administered drug, however, is excreted and thus reaches municipal wastewater. In addition, inappropriate disposal of unused drugs may further add to the burden of drugs in waste streams eventually being collected in wastewater treatment plants (WWTP). Apart from the enzyme-mediated metabolism of pharmaceutical substances in humans, or in animals in case of veterinary drugs, transformation products (TPs) can be generated by various means, including physical, chemical and biological processes.

Wastewater treatment plants, which primarily serve to reduce the content of organic matter in the waste stream, and thereby to lower the biological oxygen demand of the treated water, can be thought of as an important sink with its highly diverse pool of microorganisms degrading organic substances. Unfortunately, municipal WWTPs relying on standard conventional treatments with physical and biological stages are not optimized for complete elimination of drugs. Advanced treatment technologies, such as advanced oxidation processes, have shown great promise to enhance the removal efficiency of drugs in pilot plant but their implementation in full-scale plants is not yet widespread.

Therefore, effluent discharges from WWTPs into natural water bodies contain trace amounts of a multitude of drugs at concentrations in the ng/L to μg/L range. Despite the relatively low amounts, chronic drug exposure may compromise the health of aquatic organisms in contaminated habitats. In

addition to the presence of pharmaceuticals in domestic wastewater, pharmaceutical compounds in water bodies may also originate from landfill run-off and from disposal of animal wastes, particularly from units with intensive farming practices where the use of drugs for the prevention of disease spreading is commonplace.

In one way or another, pharmaceuticals, their human metabolites, and their TPs are in permanent contact with biota inhabiting natural waters. Pharmaceuticals dissolved in water may enter fish through the skin and the gills, and can be taken up in the bound state through the diet. Similarly to the distribution and elimination of drugs in the human body, drugs can partition into the various organs and tissues with distinct profile and can be transformed into metabolites. Taking into account that drug molecules are initially designed to interact with specific receptors through a number of molecular interactions the function and structure of exposed fish may be altered.

In the present PhD thesis, analytical methods were developed for the determination of human pharmaceuticals in muscle fish tissue, including the comparison and optimization of different types of extractions. The cleanup of the extracts was also optimized. For detection and quantification, liquid chromatography coupled to high resolution mass spectrometry on a QToF instrument, in Full MS and SWATH mode was used. The method validations covered up to 47 pharmaceuticals in targeted analysis from 14 therapeutic classes. Additionally, suspect screning analysis using a list with more tha 500 pharmaceuticals, metabolites, drugs of abuse, and TPs was performed.

The two validated analytical methodologies used either QuEChERS extraction or ultrasound extraction. In the former, a solid-liquid extraction was followed by separation of the organic solvent from the aqueous phase by salting-out. The cleanup of the extracts aimed at removing lipidic material, which may constitute a considerable fraction of fish tissue. Coextracted lipids were eliminated by specific dispersive-type adsorbents, namely EMR-lipid removal and Z-Sep adsorbent.

It took advantage of the so-called SWATH acquisition mode of the used QToF-MS instrument in a Sciex X500R, where ultra-fast data recording alternates between MS-only mode for the detection of molecular ions with up to ten sequential SWATH windows acquisitions, each of which covering an m/z 90-100 Da - wide window.

The validations of the overall methods included the parameters recovery, precision, matrix effect, reproducibility, sensitivity, robustness, and linearity. The first validated method performed with QuEChERS extraction, developed for freeze-dried fish muscle matrices, at 3 different levels of concentration up to 500 ng/g fish dw was applied to 16 samples collected from two different rivers in Italy and Greece. The quantitative analysis revealed the presence of of sotalol, carbamazepine, trimethoprim, ketoprofen, acetaminophen, propranolol and venlafaxine at concentrations of as high as 80 ng/g of fish (dw).

Unlike the first method with its broad yet limited number of analytes, the second validated methodology included also a suspect screening analysis. In this method, fish fresh samples were examined for the presence of 47 pharmaceuticals in targeted mode validated at 3 levels of concentration up to 50 ng/g of fish fw. Complementary to the validated method, more than 500 pharmaceuticals, abuse drugs, metabolites, and TPs were included for analysis by suspect screening. Positive findings were confirmed by considering both molecular ions and characteristic fragment ions supported by the instrumental library database, that includes the SCIEX All-In-One HR-MS/MS library and also the NIST 2017 library bundle, that compile spectra in high resolution for more than 17000 compounds. Confirmation was also supported using the free online chemical database ChemSpider connected with the analytical SCIEX O.S. software.

Application of this method for 32 fish samples from 4 European rivers: Italy (Adige), Greece (Evrotas), Spain (Llobregat), and a transboundary river crossing Slovenia, Croatia, Bosnia and Herzegovina, and Serbia (Sava); resulted in the detection of bezafibrate, caffeine, carbamazepine, clarithromicin, diltiazem, furazolidone, ketoprofen, sulfapiridine, trimethoprim and verapamil up to a maximum concentration of 69 ng/g of fish. Using suspect screening, benzoylecgonine, cocaine, nicotine, and ofloxacin were successfully detected and confirmed in the analyzed samples.

A further aspect of this thesis dealt with investigating the organ-specific distribution of drugs in native freshwater fish collected from the Llobregat river in NW Spain. Detection, and where possible, quantification, addressed ten biofluids, organs, and tissues. The processing of gills, bile, brain, heart, liver, muscle, pancreas, skin, plasma, and kidney from 12 samples belonging to four prominent species of the lower section of the river near Barcelona was followed by targeted and suspect screening analysis. In addition, the river water was monitoring for the occurrence of drugs.

While in the water samples, 53 substances were identified by either targeted or suspect analysis, as many as 34 compounds were detected in one or several organs of each species. Semi-quantitative assessment suggested preferential distribution of the pharmaceuticals, drugs of abuse, metabolites and TPs into kidney, skin, liver, brain and pancreas.