SUSPECT SCREENING WORKFLOW TO EVALUATE EXPOSURE AND UPTAKE OF PHARMACEUTICALS IN FISH BY UPLC-HRMS.

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ABSTRACT

The presence of pharmaceuticals residues in the aquatic environment has caused concerns about potential adverse effects on exposed wildlife. Many of the pharmaceuticals are considered as being biological active compounds capable to act as endocrine disruptors. Several studies have been reported the occurrence of pharmaceuticals in different fish tissues, but still very little is known about their disposition in fish. Several findings shows a concentration-related accumulation of drugs in different organs examined. Furthermore, fish are known to possess hepatic detoxification systems, which are likely capable of metabolizing pharmaceuticals taken up from polluted waters, and recent studies have reported the identification of different pharmaceutical metabolites. Taking advantage of the concentrations of pharmaceuticals or their metabolites in fish bile could be much higher than the concentration found in the surrounding surface waters, some studies proposed fish bile analysis for a rapid evaluation of pharmaceutical exposure. Nowadays, due to recent improvements in the instrumental analysis, the high sensitivity of accurate mass measurements by ultra performance liquid chromatography coupled to high resolution mass spectrometry (UPLC-HRMS) allow the development of screening methods allowing the detection of large number of pollutants without prior selection of compounds. In this context, suspect screening of drugs and their metabolites was performed in fish bile and muscle to evaluate the exposure and uptake of pharmaceuticals in fish along the river. A sampling campaign was done in the Llobregat river, a highly polluted Spanish river basin. A total of 75 samples of two different fish species (Barbus graellsi and Cyprinus carpio) were collected from five sampling points. Bile and muscle samples were analyzed using different analytical methodologies. First, for the rapid evaluation of pharmaceutical exposure in fish, their bile samples were analyzed directly by UPLC-HRMS after dilution with acetonitrile/0.1N hydrochloric acid (1:3). Second, in order to evaluate the accumulation of drugs in fish, muscle samples were extracted with 8 mL of methanol/0.1M acetic acid by sonication and analyzed also by UPLC-HRMS. The HRMS analysis of pharmaceuticals and their metabolites was carried out on the Q Exactive (Orbitrap)-MS system with MS/MS capability. High-quality MS/MS spectra were produced by data-dependent acquisitions. For suspect screening of pharmaceuticals and their metabolites, elemental compositions and structures were used to create a database of exact ion masses for the expected protonated or deprotonated molecules. Metabolites were included manually and using different transformation prediction softwares. In addition, software created for the management of mass spectral data provided the possibility for the detection of phase II metabolites through fragment ion search. The accurate mass measurements obtained by HRMS allowed screening for suspected drugs and their metabolites. After accurate mass extraction of expected ions, specific information such as isotopic pattern, MS/MS spectra and retention times allowed to propose plausible identities for each compound. Sometimes this information was found in libraries or predicted also by different software. Results are presented in accordance with proposed system by Schymanski *et al*. (6) of five levels for the identification confidence. With this approach several phase I metabolites, corresponding to different hydroxylation reactions, and phase II metabolites such as glucuronide and taurine conjugates were tentatively identified. The suspect analysis of muscle allowed the detection of more than ten different pharmaceuticals. Psycho-active drugs were one of the most commonly detected drugs. Their

identities were proposed by matching their accurate MS and MS/MS data against different libraries and, if available, reference standards were used to obtain the first level of confirmation of the compound identity. The analysis of fish bile has allowed the detection of plausible phase I and phase II metabolites but the identification of most of them still requires confirmation. Currently, the identification of metabolites remains a tedious and time consuming task. The lack of comprehensive MS/MS libraries hampers the metabolite identifications. Researchers must use the combination of different software, user-built libraries and literature information to propose some plausible identities. The positive findings suggested that the occurrence of drug metabolites in bile can be used as a surrogate for exposure of fish to pharmaceuticals. The analysis of muscle has allowed the detection of several pharmaceuticals in fish muscle and demonstrated the uptake of drugs from water and their plausible accumulation. The use of publicly available LC-HRMS databases for the identification of pharmaceuticals was in this case helpful. In accordance with the literature, psycho-active drugs have been the most commonly detected drugs in fish. Thus this approach highlights that public HRMS libraries of environmental contaminants and their related compounds help to mine the data in an efficient and comprehensive fashion but are still limited.

Keywords: pharmaceuticals, metabolites, suspect screening, HRMS

1. Introduction

The presence of pharmaceuticals residues in the aquatic environment has caused concerns about potential adverse effects on exposed wildlife. Many of the pharmaceuticals are considered as being biological active compounds capable to act as endocrine disruptors. Several studies have been reported the occurrence of pharmaceuticals in different fish tissues (1), but still very little is known about their disposition in fish. Several findings shows a concentration-related accumulation of drugs in different organs examined. Furthermore, fish are known to possess hepatic detoxification systems, which are likely capable of metabolizing pharmaceuticals taken up from polluted waters, and recent studies have reported the identification of different pharmaceutical metabolites (2-4). Taking advantage of the concentrations of pharmaceuticals or their metabolites in fish bile could be much higher than the concentration found in the surrounding surface waters, some studies proposed fish bile analysis for a rapid evaluation of pharmaceutical exposure (2).

Nowadays, due to recent improvements in the instrumental analysis, the high sensitivity of accurate mass measurements by ultra performance liquid chromatography coupled to high resolution mass spectrometry (UPLC-HRMS) allow the development of screening methods allowing the detection of large number of pollutants without prior selection of compounds. In this context, suspect screening of drugs and their metabolites was performed in fish bile and muscle to evaluate the exposure and uptake of pharmaceuticals in fish along the river.

2. Materials and methods

A sampling campaign was done in the Llobregat river, a highly polluted Spanish river basin (5). A total of 75 samples of two different fish species (Barbus graellsi and Cyprinus carpio) were collected from five sampling points. Bile and muscle samples were analyzed using different analytical methodologies. First, for the rapid evaluation of pharmaceutical exposure in fish, their bile samples were analyzed directly by UPLC-HRMS after dilution with acetonitrile/0.1N hydrochloric acid (1:3). Second, in order to evaluate the accumulation of drugs in fish, muscle samples were extracted with 8 mL of methanol/0.1M acetic acid by sonication and analyzed also by UPLC-HRMS. The HRMS analysis of pharmaceuticals and their metabolites was carried out on the Q Exactive (Orbitrap)-MS system with MS/MS capability. High-quality MS/MS spectra were produced by data-dependent acquisitions. For suspect screening of pharmaceuticals and their metabolites, elemental compositions and structures were used to create a database of exact ion masses for the expected protonated or deprotonated molecules. Metabolites were included manually and using different transformation prediction softwares. In addition, software created for the management of mass spectral data provided the possibility for the detection of phase II metabolites through fragment ion search.

3. Results and discussion

The accurate mass measurements obtained by HRMS allowed screening for suspected drugs and their metabolites. After accurate mass extraction of expected ions, specific information such as isotopic pattern, MS/MS spectra and retention times allowed to propose plausible identities for each compound. Sometimes this information was found in libraries or predicted also by different software. Results are presented in accordance with proposed system by Schymanski *et al*. (6) of five levels for the identification confidence. With this approach several phase I metabolites, corresponding to different hydroxylation reactions, and phase II metabolites such as glucuronide and taurine conjugates were tentatively identified. The suspect analysis of muscle allowed the detection of more than ten different pharmaceuticals (Table 1). Psycho-active drugs were one of the most commonly detected drugs. Their identities were proposed by matching their accurate MS and MS/MS data against different libraries and, if available, reference standards were used to obtain the first level of confirmation of the compound identity.

Table1: Pharmaceuticals detected in fish muscle from Llobregat river

Rt	Measured ion mass	Plausible formula	Error (ppm)	Plausible identity	Identification confidence level
4.2	205.1225	C13H17O2-	1.1	Ibuprofen	Level 1 (authentic standard)
3.5	237.1024	C15H13N2O+	0.6	Carbamazepine	Level 1 (authentic standard)
4.4	325.1716	C20H22N2OF+	1.6	Citalopram	Level 1 (authentic standard)
3.8	278.2117	C17H28NO2+	0.9	Venlafaxine	Level 2 (MassBank)
3.4	240.1595	C13H22NO3+	0.5	Salbutamol	Level 2 (MassBank)
5.4	306.0811	C17H18NCl2+	0.1	Sertraline	Level 2 (MassBank)
2.9	296.1261	C16H17NOF3+	1.7	Norfluoxetine	Level 2 (MassBank)
5.2	310.1411	C17H19NOF3+	-0.8	Fluoxetine	Level 2 (MassBank)
3.3	299.1748	C18H23N2O2+	-1.8	Carazolol	Level 2 (HRAM Compound Library - Thermo)
5.4	249.1489	C15H21O3-	1.5	Gemfibrozil	Level 2 (HRAM Compound Library - Thermo)

4. Conclusions

The analysis of fish bile has allowed the detection of plausible phase I and phase II metabolites but the identification of most of them still requires confirmation. Currently, the identification of metabolites remains a tedious and time consuming task. The lack of comprehensive MS/MS libraries hampers the metabolite identifications. Researchers must use the combination of different softwares, user-built libraries and literature information to propose some plausible identities. The positive findings suggested that the occurrence of drug metabolites in bile can be used as a surrogate for exposure of fish to pharmaceuticals. The analysis of muscle has allowed the detection of several pharmaceuticals in fish muscle and demonstrated the uptake of drugs from water and their plausible accumulation. The use of publicly available LC-HRMS databases for the identification of pharmaceuticals was in this case helpful. In accordance with the literature, psycho-active drugs have been the most commonly detected drugs in fish. Thus this approach highlights that public HRMS libraries of environmental contaminants and their related compounds help to mine the data in an efficient and comprehensive fashion but are still limited.

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