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Database-driven screening of South African surface water and the targeted detection of pharmaceuticals using liquid chromatography - High resolution mass spectrometry☆

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ABSTRACT

Pharmaceuticals and personal care products are released into aquatic environments, largely as a result of ineffectual removal during wastewater treatment. Here we present a screening strategy based on the use of three commercially available mass spectral databases, combined into a single searchable entity and parallelized by cluster computing. In addition to this, a targeted solid phase extraction method with Ultra High Pressure Liquid Chromatography coupled to quadrupole time of flight mass spectrometry (UHPLC-QTOF) was used to quantify 99 pharmaceuticals in South African surface water on a national level. Limits of quantification were in the low ng/L range for the majority of the compounds and it was found that nationally both Lamotrigine and Nevirapine occurred most often. Prednisolone and Ritonavir were present at the highest average concentration; 623 and 489 ng/L respectively. It is however shown that more than 50% of the targets chosen for analysis are not detectable in any of the samples, which highlights the utility of untargeted, database driven screening; prior to the use of costly analytical standards. Untargeted screening detected 45% of the compounds detected in targeted mode, and furthermore tentatively identified a total of 4273 unique compounds across the samples. Automatically triggered MS/MS analyses yielded 92 unique hits with greater than 95% confidence. It is therefore suggested that untargeted screening should precede the targeted approach as a matter of economy and to guide the selection of targets for quantification. There is however great room for improvement in current commercial database search methodologies as a large bottleneck exists due to processing time.

1. Introduction

The presence of pharmaceuticals in surface and drinking water has been well established in the literature (Boorman, 1999; Papageorgiou et al., 2016; Rodriguez-Mozaz et al., 2015; Wode et al., 2015; Zhang et al., 2015), with the earliest reports arising in the 1970's (Tabak and Bunch, 1970). These compounds are present in the environment largely due to excretion by humans and livestock that have ingested them. Other routes of surface water contamination that should be considered are pit latrines (Graham and Polizzotto, 2013), improper pharmaceutical destruction (Peng et al., 2014), malfunctioning sewage treatment plants and illegal sewage disposal. Many of these factors are unique to the African continent and other "developing regions" and are not sufficiently addressed in the literature.

The presence of pharmaceuticals in the environment has been proven worldwide, yet little work has been done in South Africa and Africa as a whole on this topic. The "African picture", which is unique in that many regions are water scarce and people often utilize unpurified water for drinking, has been largely neglected. This has been established through searching the curated USEPA

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database (Daughton and Scuderi, 2012) on this field. It is heartening though that missing pieces of the picture are now being added regularly by researchers across the African continent (Agunbiade and Moodley, 2014, 2016; Schoeman et al., 2015; K'oreje et al., 2016).

Since South Africa faces a number of unique challenges such as: water scarcity, malfunctioning waste water treatment, HIV burden, a high Tuberculosis (TB) prevalence, TB drug resistance and reduced access to fresh water; this topic is extremely relevant to the population as a whole. The behavior and fate of these compounds in the environment has yet to be established completely in the literature (la Farré et al., 2008; Miège et al., 2009; Padhye et al., 2014; Westerhoff et al., 2005) and it is believed that their presence in the environment could contribute to drug resistance (Gatica and Cytryn, 2013; Jain et al., 2013; Khetan et al., 2007; Kümmerer, 2009). This is especially important given the TB challenges faced in South Africa.

Ferrer and Thurman (2012) utilized liquid chromatography coupled to high resolution mass spectrometry to detect 100 pharmaceuticals in surface water. The researchers highlighted the utility of accurate mass analysis in this field. Isomers do however exist for chemicals of interest necessitating the use of chemical standards, which could hopefully be distinguished chromatographically or by mass spectral fragmentation. High resolution mass spectrometry is rapidly gaining popularity in the field of micropollutant detection and researchers are able to screen for an ever increasing number of targets (Alygizakis et al., 2016; Bletsou et al., 2016; Cotton et al., 2016; Gago-Ferrero et al., 2016; González-Mariño et al., 2016; Soulier et al., 2016).

Similarly high resolution mass spectrometry is being used to determine the environmental fate of pharmaceuticals and their transformation as a result of wastewater treatment. The technology allows researchers to detect and characterize novel disinfection transformation products with a high level of sensitivity (Boix et al., 2016; Ibáñez et al., 2016; Rager et al., 2016; Wood et al., 2016). This means that if even though the original parent molecule is not detectable in a sample, the detection of its transformation products allows researchers to draw inferences relating to its prevalence in wastewater treatment works (WWTW) systems.

High resolution mass spectrometric instrumentation is improving rapidly and what once was used only for characterization now allows one to perform sensitive quantification studies. Since the majority of pharmaceutical compounds are ionized and measured, the technology yields a broader picture of a particular sample (Glauser et al., 2016).

Having had success with the detection of antiretrovirals (ARVs) (Wood et al., 2015) in surface water, the research is followed by this work to include a broader variety of compounds. Samples have been taken from across South Africa from almost all the major rivers and dams (man-made) in the country. This has led to the development of an important data resource which is the establishment of a national baseline for these types of compounds. By screening this type of sample using high resolution mass spectrometry one is not limited by the pharmaceutical standards at one's disposal to use for targeted comparisons; the data may be mined in various ways to identify targets for validation at a later stage.

Some of the drawbacks of LC-MS analysis are difficulties of inter-laboratory reproducibility (Rivier, 2003) as well as a lack of a comprehensive cross-platform database. With high resolution instruments producing accurate mass values for unknowns, data can now be compared more easily between laboratories. In addition to this a number of commercial databases have become available for a variety of compounds. The size of the high resolution mass spectral data files and the current algorithms used in comparing them against even small databases (± 80 000 compounds) is at this time proving cumbersome, with a single LC-MS data file search routine taking up to 12 h. For this reason we investigate the utility of massively parallel cluster computing to alleviate the computational bottleneck. The advantage of this approach is that the user interface remains the same, only that a single user is able to process multiple iterations of a software package simultaneously from a single interface. This allows a user that is only familiar with the mass spectral software to process samples without necessitating further training on aspects of cluster computation.

In this work we describe not only the prevalence of these compounds but also the analytical procedure, which involves three distinct injections of the same sample, each with its own mass spectral acquisition mode. Targeted screening in Full Scan mode without fragmentation, which provides unfragmented pseudomolecular ion information, is followed by untargeted screening in which high abundance ions are automatically selected for fragmentation (Auto MS/MS), subjected to compound dependent collision energy, yielding tandem mass spectra. In the third method, low abundance ions, that are missed by Auto MS/MS selection are fragmented by applying a fixed collision energy to all the ions detected by the instrument (All Ions mode, no precursor ions selected). This yields fragmentation information relatable to the complementary data generated in the Full Scan approach via the accurate masses (and elemental composition of ions) available from both runs at identical retention times. With this methodology, the maximum amount of information can be gleaned from the data set. The data set also has a certain amount of longevity in that it can be re-mined as new targets and scientific questions arise.

2. Materials and methods

2.1. Chemicals and reagents

Analytical reference standards were purchased from Toronto Research Chemicals (Toronto, Canada) the US, British and European Pharmacopoeia (Industrial analytical, Johannesburg, South Africa), and Sigma Aldrich (Johannesburg, South Africa). The reference standards were all 97% pure or better as indicated by the manufacturer.

Target compounds were dissolved in dimethyl sulfoxide (DMSO), to 1 mg/mL, and stored at −20 °C until use. Standards were grouped into five sets according to molecular mass to ensure no isobars were present in the same set and diluted to 10 μg/mL in DMSO. The sets were stored at −20 °C until use and combined to produce the appropriate working solutions. ^{13}C3-trimethyl caffeine from Cambridge isotope Laboratories (Tewksbury, MA, USA), diluted to 10 μg/mL in methanol immediately prior to use. Concentrated analytical reference compounds were handled and prepared in a separate designated area to avoid contaminating environmental samples or other consumables. Acetonitrile, methanol, DMSO and water (LC-MS grade) were obtained from Lab-Scan (Gliwice, Poland). Premixed water and acetonitrile (0.1% formic acid) were obtained from Burdick and Jackson (Milwaukee, MI, USA). No South African water was for reagent purposes to avoid the possible introduction of contaminants into the analytical process.

2.2. Method optimization

36 of the target compounds were randomly selected from the 5 pharmaceutical groups and diluted in DMSO to 1 μg/mL and injected in triplicate (1 ng on column) to compare the chromatographic characteristics of six columns, namely the: Zorbax Eclipse XDB C8 RRHD 4.6 × 150 mm, 1.8 μm; Zorbax Eclipse XDB C8 RRHT 2.1 × 100 mm, 1.8 μm; Poroshell 120 SB-C8 2.1 × 100 mm, 2.7 μm;