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The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water

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ABSTRACT

The study and quantification of personal care products, such as pharmaceuticals, in surface water has become popular in recent years; yet very little description of these compounds' presence in South African surface water exists in the literature. Antiretrovirals (ARVs), used to treat human immunodeficiency virus (HIV) are rarely considered within this field. A new method for the simultaneous quantification of 12 antiretroviral compounds in surface water using the standard addition method is described. Water samples were concentrated by a generic automated solid phase extraction method and analysed by ultra-high pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Substantial matrix effect was encountered in the samples with an average method detection limit of 90.4 ng/L. This is the first reported countrywide survey of South African surface water for the quantification of these compounds with average concentrations ranging between 26.5 and 430 ng/L.

1. Introduction

Concerns regarding the presence of personal care products (PCPs), such as pharmaceuticals, in water supplies have arisen recently with various researchers showing that a wide variety of pharmaceuticals are discharged into the environment as a result of inadequate wastewater treatment (Ferrer and Thurman, 2012; Yu et al., 2010; Luo et al., 2014). This appears to be a global phenomenon (Kümmerer, 2009), and in addition to discharge from wastewater treatment works (WWTWs) one should also consider alternative sources of contamination such as improper disposal of expired pharmaceutical stocks e.g. leachate from pharmaceutical landfilling (Peng et al., 2014a, 2014b), or, pit latrines (Graham and Polizzotto, 2013) in developing countries. There is a marked gap in the literature, regarding this global phenomenon, describing the situation in Africa.

Very little research has been carried out in South Africa to determine the presence of pharmaceuticals and their degradation products in surface water using mass spectrometry; as determined by searching the curated "US EPA Bibliographic Database of Publications Relevant to Pharmaceuticals and Personal Care Products" (Daughton and Scuderi, 2012). Also, South Africa utilises more antiretroviral compounds per capita than any other nation in the fight against HIV/AIDS, with approximately 2 150 880 people receiving ARVs in 2012 as contrasted to the approximate 199 000 people on ARV therapy in Eastern Europe (WHO, 2013). This presents a novel problem with regards to the presence and transformation of these compounds in the environment. Since South Africa uses more of these compounds than any other nation it has been theorized that these compounds should be present in the environment to a much greater extent. This phenomenon should also be exacerbated by the overall low rainfall and water scarcity in sub-Saharan Africa; which would lead to lower environmental dilution of the target compounds.

Anti-HIV compounds such as nucleoside and non-nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, entry inhibitors and integrase strand transfer inhibitors, are used to treat HIV ("FDA Antiretroviral drugs used in the treatment of HIV infection," 2014) and to prevent mother-to-child transmission (Mofenson, 2010). The breadth of the compound class therefore poses an interesting analytical challenge, and to our knowledge no other research addressing their simultaneous

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detection, in any environmental matrix, has been carried out.

Prasse et al. (2010) studied the presence of five anti-HIV compounds in addition to other anti-virals in the Hessian Ried river systems and found their presence as a result of WWTW discharge. Peng et al. (2014a, b) utilised a similar methodology to detect antiviral drugs, including Stavudine and Zidovudine, in the Pearl River Delta in China; but could not detect these compounds in surface water. Given the global usage of these pharmaceuticals and since the compounds have been detected in European surface water and WWTW influent and effluent, it is predicted that higher concentrations should be present in South African water supplies due to higher usage in the population. These compounds can be seen as additional candidates for consideration as emerging pollutants.

The main objective of this work was to develop a single LC-MS/MS method for the analysis of 12 commonly used anti-HIV compounds, concentrated by generic solid phase extraction (SPE), to quantitatively determine their prevalence in South African surface water. This work also represents the first step in a nationwide survey for the detection of pharmaceuticals in surface water in South Africa.

2. Materials and methods

2.1. Chemicals and reagents

Analytical reference standards obtained from the US, British and European Pharmacopoeia as well as Toronto Research Chemicals (Toronto, Canada) were purchased from Industrial Analytical (Johannesburg, South Africa). All compounds were of 97% purity or better as indicated by the vendor. Zalcitabine, Tenofovir, Abacavir, Efavirenz, Lamivudine, Didanosine, Stavudine, Zidovudine, Nevirapine, Indinavir, Ritonavir, Lopinavir and caffeine stock solutions (1 mg/mL) were prepared in methanol and stored at −20 °C until use. 13C3-trimethyl caffeine, 100 μg/mL in methanol, was obtained from Cambridge isotope Laboratories (Tewksbury, MA, USA) and diluted to 20 μg/mL in methanol before use. Standards were prepared and handled in a separate room from samples in order to prevent cross contamination. LC-MS grade acetonitrile, methanol and water were purchased from Lab-Scan (Gliwice, Poland) and formic acid and ammonium formate from Merck (Johannesburg, South Africa). No South African-origin water was used as a reagent in the course of this research.

2.2. Environmental sample collection and extraction

Grab samples were collected from various surface water sources in South Africa, detailed in Table S1 (Supporting Information). Sampling locations were selected based on their proximity to WWTWs and the distance from major bodies of water. None of the samples were part of a “pristine” water course and all were either downstream of WWTWs or urbanised environments. Samples were collected in “virgin” borosilicate Schott bottles while wearing nitrile gloves (to prevent the introduction of contaminants) and transported, protected from light, to the laboratory at room temperature. Samples were stored at −20 °C until extraction, after which extracts were stored at −20 °C until analysis.

500 mL of each sample was filtered using a 1 μm glass-fibre syringe-driven filter (Pall, USA) and extracted using the Smart Prep Extraction System (Horizon, USA), which is an automated offline solid phase extraction instrument. The extraction procedure was modified from a method developed by Ferrer and Thurman in order to maintain a level of universality (Ferrer and Thurman, 2012). Briefly, 6 cc Oasis HLB, 500 mg SPE cartridges (Waters, Milford, MA, USA) were conditioned with 4 mL of methanol followed by 6 mL of HPLC-grade water. 500 mL of sample was introduced at a flow rate of 10 mL/min after which cartridges were dried under nitrogen for 3 min. Cartridges were eluted twice with 5 mL of methanol and the eluate dried under a gentle stream of nitrogen to 500 μL. All extractions were performed at 18 °C (±0.5 °C) in a dedicated area.

The standard addition method was used to quantify all target analytes, with modification from the traditional approach (Conley et al., 2008). Each extracted sample (190 μL) was combined with 10 μL 13C3-caffeine standard and divided into four aliquots of 45 μL each. To these, 5 μL of either 10 000 ng/mL, 1000 ng/mL, 100 ng/mL or 0 ng/mL standard mixture in methanol was added. The samples were analysed in triplicate in order of increasing concentration, with blank injections between each in order to prevent and evaluate carry-over. Standard addition data was analysed using Mass Hunter Quant (Agilent, Santa Clara, USA). The data obtained from these analyses were compared to an external calibration curve, generated by injecting a mixture of standards in methanol at 1, 10, 100 and 1000 ng/mL, in order to assess the effects of the matrix. Retention time reproducibility was checked periodically by injecting a standard mixture after every batch analysis.

2.3. LC-MS/MS analysis

An array of similar chromatographic columns were tested to optimise separation. The XDB-C8 1.8 μm 3.0 × 50 mm, Eclipse Plus RRHD C18 1.8 μm 2.1 × 50 mm (Agilent), Kinetex PFP 1.7 μm 50 × 2.1 mm, Kinetex XB-C18 1.7 μm 50 × 2.1 mm, Kinetex C8 2.6 μm 50 × 4.6 mm and Kinetex C18 1.7 μm 50 × 2.1 mm (Phenomenex) were evaluated. Combinations of mobile phases such as water and either acetonitrile or methanol with either 0.1% formic acid or 5 mM ammonium formate were tested, to lead to the optimized method. Extensive method validation was carried out and may be found described further in the Supplementary information.

SPE extracts were analysed by LC-ESI-MS/MS. Target compounds were separated using an Agilent 1290 series UHPLC and mobile phases consisted of water (A) and acetonitrile (B) both with 0.1% formic acid. Following a 15 μL injection onto a Zorbax Eclipse C8 XDB, 3.0 × 50 mm, 1.8 μm column, the chromatographic gradient was as follows: 0% B, 3 min; 100% B 20 min; 100% B 25 min; 0% B 30 min; 0% B 40 min. A flow rate of 0.4 mL/min was used, and the column was maintained at 22 °C with no column effluent splitting. The UHPLC was coupled to an Agilent 6460 triple quadrupole, equipped with a Jet Stream electrospray ionization (ESI) source. All analyses were performed in positive ion mode.

MS/MS optimisation was performed automatically using the Agilent Optimizer software package (Table 1). These settings were then combined into a single dynamic MRM method with the following ESI Jet Steam source conditions: Delta EMV 400 V, gas temperature 250 °C, gas flow 8 L/min, nebuliser pressure 35 psi, sheath gas temperature 300 °C, sheath gas flow 10 L/min and capillary voltage 3000 V.

3. Results and discussion

3.1. Chromatography and mass spectrometry

A variety of chromatographic programs, columns and solvents were tested; yet it was decided to sacrifice optimal conditions for the sake of universality. A number of the compounds are polar and were not effectively retained on C18 columns. A short C8 column (50 mm) was therefore chosen in order to retain more polar compounds and reduce elution times of non-polar compounds. A