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Development of a simple analytical method for the simultaneous determination of paracetamol, paracetamol-glucuronide and *p*-aminophenol in river water

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ABSTRACT

Paracetamol is among the most worldwide consumed pharmaceuticals. Although its occurrence in the environment is well documented, data about the presence of its metabolites and transformation products is very scarce. The present work describes the development of an analytical method for the simultaneous determination of paracetamol, its principal metabolite (paracetamol-glucuronide) and its main transformation product (*p*-aminophenol) based on solid phase extraction (SPE) and high performance liquid chromatography coupled to diode array detection (HPLC-DAD). The method was applied to analysis of river waters, showing to be suitable to be used in routine analysis. Different SPE sorbents were compared and the use of two Oasis WAX cartridges in tandem proved to be the most adequate approach for sample clean up and pre-concentration. Under optimized conditions, limits of detection in the range 40–67 ng/L were obtained, as well as mean recoveries between 60 and 110% with relative standard deviations (RSD) below 6%. Finally, the developed SPE-HPLC/DAD method was successfully applied to the analysis of the selected compounds in samples from seven rivers located in the north of Portugal. Nevertheless all the compounds were detected, it was the first time that paracetamol-glucuronide was found in river water at concentrations up to 3.57 μg/L.

1. Introduction

Paracetamol (acetaminophen or *N*-acetyl-4-aminophenol) is one of the most popular and widely used medicines for the treatment of pain and fever, both as an over-the-counter (OTC) and as a prescribed medicine. It can be used in a wide range of patients, including children, pregnant women or the elderly. Following oral administration, approximately 90% of paracetamol is metabolized, being conjugated with glucuronide (40–67%) and, in a less extent, with sulphate (20–46%), to form inactive metabolites, which are eliminated in urine together with a small fraction of unchanged paracetamol (<5%) [1]. Although paracetamol presents a high removal efficiency (approximately 99%) in WWTPs [2,3], it has been detected in their effluents at concentrations up to low microgram per litre [4–6], contributing to its entrance into surface waters [7–9]. Once in the environment, paracetamol is mainly degraded by microorganisms, which are capable of using it as carbon and energy sources [10].

Although paracetamol is not highly persistent in the environment, continuous input overrules its high transformation rate [11], thus, it can adversely affect aquatic organisms. Acute toxicity effects in the invertebrate *Daphnia magna* (EC_{50} ranging from 26.6 to 50 mg/L) [12–14], the marine bacterium *Vibrio fischeri* (EC_{50} = 549.7 mg/L) and the fish *Oryzias latipes* (EC_{50} = >160 mg/L) [12] have been reported. Effects on cell cultures with EC_{50} values of 19 mg/L have also been described [13].

Nowadays analytical methodologies described in literature are mainly focused in multi-residues methods that allow the simultaneously determination of paracetamol together with a large number of pharmaceuticals from several therapeutic groups [15–17]. Most of them are principally focused in parent compounds and rarely analyze metabolites and/or transformation products. At present, methods reported for the determination of paracetamol metabolites are focused in biological matrices [18–20] rather than

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in environmental ones [21]. On the other hand, paracetamol may also be degraded, both during wastewater treatment and in the environment, giving different transformation products [22,23]. p-Aminophenol was identified as its main transformation product, and its presence in wastewater samples was reported [23]. However the origin of *p*-aminophenol cannot only be attributed to the degradation of paracetamol, since it is also widely used in industrial applications and is known as a transformation product from pesticides. Furthermore, *p*-aminophenol was also described as the primary degradation product of paracetamol during the storage of its medicinal formulations [24].

High-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) has been designated as technique of choice for the determination and quantification of pharmaceuticals in environmental samples [25]. However, these equipments are still very expensive and they are not available in many laboratories for routine analysis. On the other hand, almost all laboratories have HPLC systems with diode array UV absorbance and/or fluorescence detection that may effectively be used for the analysis of pharmaceuticals in environmental samples [26–29].

Due to the complexity of environmental samples, analysis of pharmaceuticals has to be preceded by a pre-concentration step, which allows the detection of low concentrations and simultaneously removes the interferences. This is often performed by solid phase extraction (SPE). Generally, polymeric sorbents, like Oasis HLB, are the most used for pre-concentration of pharmaceuticals from aqueous matrices [16,17,30–32], though mixed-mode ion-exchange sorbents have also been described [33,34].

Thus, the present work describes the development and validation of an analytical method based on off-line SPE, using a mixed mode reversed phase/anionic exchange sorbent, followed by LC-DAD for the determination of paracetamol, its main metabolite (paracetamol-glucuronide) and its principal transformation product (*p*-aminophenol) in river waters. The performance and application of this method is important, since allows the simultaneous monitoring of parent compound, metabolite and transformation product as well as the evaluation of their environmental interdependence, using one of the most worldwide consumed pharmaceuticals (paracetamol) as example.

Finally, the developed methodology was successfully applied to the analysis of the selected compounds in seven rivers from north of Portugal. To our knowledge this is the first time that paracetamol-glucuronide was found in surface waters.

2. Materials and methods

2.1. Chemicals and reagents

Paracetamol (PCT) (acetaminophen), *p*-aminophenol (PAP) (4-aminophenol) and paracetamol-glucuronide (PCT-G) (ρ-acetamidophenyl β-D-glucoronide) sodium salt were purchased from Sigma-Aldrich (Steinheim, Germany). All standards were of high purity grade (>93%). HPLC-grade methanol, HPLC-grade acetonitrile and HPLC-grade acetone, n-hexane and formic acid (purity ≥98%) were obtained from Merck (Darmstadt, Germany), hydrochloric acid 37% and glacial acetic acid (purity ≥99.7%) were purchased from Carlo Erba (Rodano, Italy), ammonia 25% was obtained from Panreac (Barcelona, Spain), ammonium hydroxide solution, ammonium acetate (purity ≥98%), ethyl acetate and dichloromethane were purchased from Sigma-Aldrich (Steinhein, Germany). HPLC-grade water (18.2 MΩ cm) was obtained by purifying deionised water in a Milli-Q Simplicity 185 system (Millipore, Molsheim, France).

Individual stock standard solutions were prepared for each compound by dissolving 10 mg of powder in 10 mL of methanol, obtaining a final concentration of 1000 mg/L, and stored at −20 °C. Stock standard solutions were renewed every week. An intermediate standard solution was daily prepared by mixing the three individual stock solutions and diluting with a mixture methanol–water (10:90, v/v) to give a final concentration of 10 mg/L and kept at 4 °C. Working standard solutions were also prepared in a mixture methanol–water (10:90, v/v) by dilution of appropriate amounts of the intermediate solution. Amber glassware was used to prevent light degradation. These working standard solutions were used for preparation of the calibration curve and for spiking samples in the validation study.

All standard solutions and sample extracts were filtered through a 0.20 μm PTFE syringe filter (Teknokroma, Barcelona, Spain) and homogenized using a vortex mixer (VWR, Radnor, Delaware, USA). All chromatographic solvents were filtered through a 0.20 μm nylon membrane filter (Supelco, Bellefonte, PA, USA) using a vacuum pump (Dinko D-95, Barcelona, Spain) and degassed for 15 min in an ultrasonic bath (Raypa® Trade, Terrassa, Spain).

SPE cartridges used were Oasis® MAX (60 mg, 3 mL), Oasis® WAX (150 mg, 6 mL), Oasis® MCX (150 mg, 6 mL) and Oasis® HLB (200 mg, 6 mL) from Waters (Mildford, MA, USA), LiChrolut® EN/RP-18 (EN 40–120 μm, 100 mg (bottom) and RP-18 40–63 μm, 200 mg (top), 6 ml) from Merck (Poland), Strata™-SDB-L (500 mg, 6 mL) and Strata™-X (200 mg, 3 mL) from Phenomenex (USA), and Enviro-clean® (C_8 and quaternary amine, 1000 mg, 6 mL) from Unit Chemical Technologies (UCT), Inc. (Bristol, PA, USA).

2.2. Sample collection

River water (2.5 L) was collected from seven rivers located in the north of Portugal, which is one of the most densely populated areas of the country. Sample collection, preservation and storage were done according to the US EPA Method Guideline [35]. River samples were collected on the river side in amber glass bottles and kept refrigerated (±4 °C) during the transport to the laboratory. Samples were collected along one week in September 2011.

2.3. Sample pre-treatment and extraction

River water samples were vacuum filtered through 1.2 μm glass microfiber filters (GF/C, Whatman, UK), followed by 0.20 μm nylon membrane filters (Supelco, Bellefonte, PA, USA) and stored at −20 °C, until extraction.

For the SPE procedure a vacuum manifold system (Phenomenex, USA) was used. Two Oasis WAX cartridges were initially conditioned, in separate, with 2 mL of methanol, 2 mL of HPLC-grade water, and 2 mL of HPLC-grade water pH 7 (pH adjusted with ammonia) at a flow rate of 1 mL/min. After that, the SPE cartridges were connected in tandem and 50 mL of river water (pH adjusted to 7 with ammonia) were loaded onto the cartridges at a flow rate of 1 mL/min. Finally, analytes were eluted with 5 mL of methanol and 5 mL of 5% ammonium hydroxide in methanol at a flow rate of 1 mL/min, and the eluates were pooled in one single collection vial. Extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 250 μL of a mixture methanol–water (10:90, v/v), allowing a pre-concentration factor of 200.

2.4. Liquid chromatography

Chromatographic analysis was performed on a Nexera Ultra-High Performance Liquid Chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with two solvent delivery modules LC-30 AD, a column oven CTO-20 AC, an autosampler SIL-30 AC and an UV/vis photodiode array detector SPD-M20A. The system was controlled by a system controller CBM-20A. Two chromatographic systems were used in this work in order to