Ultrasonics Sonochemistry 26 (2015) 388–398

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Effects of various factors of ultrasonic treatment on the extraction recovery of drugs from fish tissues

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ARTICLE INFO

Article history:
Received 29 August 2014
Received in revised form 1 January 2015
Accepted 6 March 2015
Available online 12 March 2015

Keywords:
Ultrasound-assisted extraction
Drugs
Fish
Chromatography

ABSTRACT

In the present research, a combined extraction method of ultrasound-assisted extraction (UAE) in conjunction with solid phase extraction (SPE) was applied to isolation and enrichment of selected drugs (metoprolol, ticlopidine, propranolol, carbamazepine, naproxen, acenocumarol, diclofenac, ibuprofen) from fish tissues. The extracted analytes were separated and determined by ultra-high performance liquid chromatography with UV detection (UHPLC–UV) technique. The selectivity of the developed UHPLC–UV method was confirmed by comparison with ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) analysis.

The important parameters, such as composition of type and pH of extraction solvent, solid/liquid rate volume of extraction solvent and number of extraction cycles were studied. The ultrasonic parameters, such as time, power and temperature of the process were optimized by using a half-fraction factorial central composite design (CCD). The mixture of 10 mL of methanol and 7 mL of water (pH 2.2) (three times) was chosen for the extraction of selected drug from fish tissues. The results showed that the highest recoveries of analytes were obtained with an extraction temperature of 40 °C, ultrasonic power of 300 W, extraction time of 30 min.

Under the optimal conditions, the linearity of method was 0.12–5.00 μg/g. The determination coefficients (R^2) were from 0.979 to 0.998. The limits of detection (LODs) and limits of quantification (LOQs) for the extracted compounds were 0.04–0.17 μg/g and 0.12–0.50 μg/g, respectively. The recoveries were between 85.5% and 115.8%.

1. Introduction

The discharge, presence and potential effects of pharmaceuticals in the environment have attracted increasing attention in recent years. Pharmaceuticals are a broad and diverse group of chemicals developed and used to produce specific biological effects in human and animal health care and livestock farming. Residues of these compounds in animal foods, including those raised in aquaculture, intended for human consumption are of toxicological and regulatory concern since the presence of these drug residues, regardless of their minute amounts, can trigger potential adverse side effects in humans such as allergic reactions in hypersensitive individuals, other long-term health effects, or they can be potential carcinogenic [1,2].

Drugs enter the environment via sources which include the effluents from wastewater treatment plants, leakage from septic tanks and landfill sites, surface water run-off from farm land, aquaculture and disposal into water courses. Municipal effluents represent a major source of pollution. Consequently, human pharmaceutical residues have been recognized as "emerging" environmental pollutants due to their near ubiquitous detection adjacent urban areas at trace or ultratrace levels; a phenomenon accelerated by rapid urban growth and aging population demographics. Continuous release of these compounds and their bioactive metabolites and degradation products within municipal wastewater discharges often results in constant low-level exposure for organisms inhabiting such receiving environments [3].

The amount of pharmaceuticals and their bioactive metabolites being introduced into the environment is probably low. However, their continual input into the environment may lead to a high, long-term concentration and promote unnoticed adverse effects on aquatic and terrestrial organisms. Effects can accumulate so slowly that changes remain undetected until they become irreversible [4]. Consequently, the presence, uptake, and bioconcentration of these pollutants by aquatic organisms should be monitored to characterize the environmental persistence and potential impact on exposed organisms. However, there is a perceived need to

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http://dx.doi.org/10.1016/j.ultsonch.2015.03.005
1350-4177/

develop efficient extraction and selective enrichment methods which may be applied to real complex matrices such as exist in living organisms.

Some methods of extraction for pharmaceutical compounds have been described. Mainly the pressurized liquid extraction (PLE) [5–10] has been used for the extraction of drugs in aquatic organisms, such as crustaceans, mussels, algae, and fish, and only in a one work the ultrasonication (UAE) UAE has been applied [11]. Regarding the crucial purification step of the sample extract, different clean-up procedures have been used: solid-phase extraction (SPE) [9–18] solid phase microextraction (SPME) [19,20] or gel-permeation chromatography (GPC) [6–8]. Last step in the analytical process includes the identification and determination of drugs, usually based on liquid (LC) [5,6,9–15,19–25] or gas chromatography (GC) [7,8,16–18] mostly in combination with mass spectrometry (MS) detection.

This work describes the development, optimization and validation of a method for the determination of eight multi-class pharmaceuticals (metoprolol, ticlopidine, propranolol, carbamazepine, naproxen, acenocumarol, diclofenac, ibuprofen) in fish tissues, selected according to their detection frequency in water and sediment in rivers as well as to their potential negative effects in aquatic organisms. The developed method is based on an extraction step using ultrasound-assisted extraction (UAE) followed by solid phase extraction (SPE) clean-up and ultra-high performance liquid chromatography with UV detection (UHPLC–UV) for the determination of target compounds. Critical steps in method development involved the selection and optimization of the most appropriate sample pre-treatment step that allowed the simultaneous extraction of selected compounds from fish tissues. The evaluation and achievement of optimum conditions based on their interaction and main effect is simply carried out using different experimental design methodology for e.g. optimization of ultrasound-assisted reverse micelles dispersive liquid–liquid micro-extraction, optimization of ultrasonic assisted adsorption of safaranin O by tin sulfide nanoparticle loaded on activated carbon, optimization of the ultrasonic assisted removal of methylene blue by gold nanoparticles loaded on activated carbon, optimization of the combined ultrasonic assisted/adsorption method for the removal of malachite green by gold nanoparticles loaded on activated carbon [26–29]. In the present study, the ultrasound-assisted extraction parameters such as time, power and temperature of process, type and pH of extraction solvent, solid/liquid rate volume of extraction solvent, number of extraction cycles and volume of water for dilution of extract before SPE were selected. Response surface methodology (RSM) was employed to optimize extraction conditions (temperature, liquid–solid ratio and duration) in order to obtain the maximal extraction efficiency.

2. Material and methods

2.1. Chemicals and reagents

High purity grade (>95%) pharmaceutical standards including: (±)-metoprolol (+)-tartrate salt (MET), ticlopidine hydrochloride (TIC), (±)-propranolol hydrochloride (PRO), carbamazepine (CBZ), naproxen (NAP), diclofenac sodium salt (DIC), ibuprofen (IBU) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acenocoumarol (ACE) was obtained from U.S. Pharmacopeia (Rockville, MD, USA). (±)-Carvedilol (CAR) (IS, internal standard) was kindly provided by Toronto Research Chemicals Inc. (North York, Canada). HPLC-grade trifluoroacetic acid (TFA), water and acetonitrile were obtained from Merck (Darmstadt, Germany). Analytical-grade methanol, acetonitrile, ethyl acetate, ammonium hydroxide, phosphoric acid and formic acid were purchased from POCH S.A. (Gliwice, Poland).

Standard stock solutions of the pharmaceuticals were prepared in methanol at a concentration of 1 mg/mL. The working solutions were prepared by appropriate dilution of the stock solutions in methanol. All solutions were stored at 4 °C in the dark.

2.2. Apparatus and chromatographic conditions

The chromatographic analysis was carried out using a UHPLC system (Merck Hitachi, Germany) combined with a pump (Model L-2160U), UV detector (Model L-2400U), autosampler (Model L-2200), thermostated column compartment (Model L-2350U) and a degasser module. The entire configuration was operated by an EZ Chrom Elite System Manager.

Chromatographic separations were carried out using a Poroshell 120 EC-C18 analytical column (100 mm × 3.0 mm; 2.7 μm, Agilent Technologies, USA) operated at 25 °C. A binary gradient consisting of (A) 0.05% trifluoroacetic acid in water and (B) acetonitrile was employed to achieve chromatographic separation and is defined in Table 1. The injected volume was 2 μL. Monitoring and quantitation were performed at 223 nm for selected drugs and for the IS.

Under these conditions, the analytes eluted with the internal standard within 6 min. The individual compounds were identified by comparing their retention time and their identification was verified by the standard addition method. The identified drugs were quantified according to respective standard calibration curves.

The confirmatory UHPLC–MS/MS analysis was performed on a Dionex UPLC system (Dionex Corporation, Sunnyvale, CA, USA) coupled with an AB Sciex Q-Trap® 4000 mass spectrometer (Foster City, CA, USA). The chromatographic separation was performed using the column and gradient elution program described above, except for the application of TFA in the mobile phase. TFA strongly suppresses ionization in the negative ion mode, and so 0.1% formic acid in water was used as a component of the mobile phase if an MS/MS detector was employed during sample analysis. MS/MS conditions were applied as described previously [30]. To optimize ESI conditions for selected drugs, quadrupole full scans were carried out in positive and negative ion detection modes. The mass spectrometric parameters were as follows: source temperature – TEM = 500 °C, ionization voltage – ISV = 4000 V or −4000 V, collision assisted dissociation – CAD = medium, curtain gas – CUR = 10 psi, sheath gas – GS1 = 90 psi and desolvation gas – GS2 = 80 psi. Analysis was performed in the MRM mode, using the precursor ions and the corresponding product ions. MRM transitions and crucial compound-dependent parameters such as declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) are listed in

Table 1
Time-scheduled gradient elution program.

Time (min)	Mobile phase composition		Flow rate (mL/min)
	A (%)	B (%)	
0.0	12	88	0.5
1.0	40	60	0.7
2.5	40	60	0.7
3.0	55	45	0.7
3.5	85	15	0.7
4.0	85	15	0.7
4.5	85	15	0.4
4.9	85	15	0.3
5.0	12	88	0.8
5.1	12	88	0.5
6.0	12	88	0.3