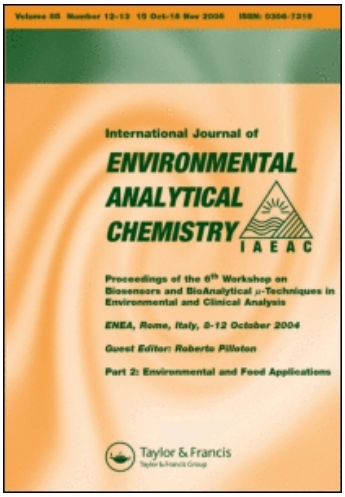

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Atenolol removal in microcosm constructed wetlands

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Online publication date: 22 September 2010

To cite this Article Dordio, Ana , Pinto, José , Dias, Cristina Barrocas , Pinto, Ana Paula , Carvalho, Alfredo J. Palace and Teixeira, Dora Martins(2009) 'Atenolol removal in microcosm constructed wetlands', International Journal of Environmental Analytical Chemistry, 89: 8, 835 — 848
To link to this Article: DOI: 10.1080/03067310902962502
URL: http://dx.doi.org/10.1080/03067310902962502

Intern. J. Environ. Anal. Chem.
Vol. 89, Nos. 8–12, 15 July–15 October 2009, 835–848

Taylor & Francis
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(*Received 31 July 2008; final version received 2 April 2009*)

Microcosm constructed wetland systems established with a matrix of light expanded clay aggregates (LECA) and *Typha* spp. or *Phragmites australis* were used to evaluate their ability to remove atenolol from wastewater. Combined with an efficient SPE concentration step, the use of HPLC-DAD yielded an analytical method for atenolol quantification with very low LOQ ($9\,ng\,mL^{-1}$) and high reproducibility (RSD < 4%). Overall removal efficiencies of 92.5% and 94.5% were achieved after a retention time of only 4 days with the microcosm systems planted with *Phragmites australis* and *Typha* spp., respectively. The removal kinetics was characterised by an initial fast step (removal of about 75% after just 24 h) which is mainly attributable to adsorption on the LECA matrix. Atenolol removal in LECA beds continues to increase in a steady pace up to the end of the assay (8 days) being nevertheless about 5–10% lower than those observed in the planted beds after the first 4 days. For the retention time of 4 days most of the atenolol is removed by the LECA matrix but an additional 12–14% of the overall removal efficiency can be attributed to the *Typha* and *Phragmites* plants, which is in agreement with other published reports. Despite the fact that further tests using larger-scale flowing systems are required to evaluate fully the atenolol behaviour in constructed wetlands, this study points to the possible application of these low-cost wastewater treatment systems to treat atenolol contaminated wastewater.

Keywords: pharmaceuticals; atenolol; subsurface flow constructed wetlands (SSFCWs); LECA; *Phragmites australis*; *Typha* spp.

1. Introduction

Contamination of aquatic systems with pharmaceutical residues has emerged recently as one of the key issues in environmental chemistry. Analytical techniques made available in the past few years have significantly lowered the detection and quantification limits for organic substances in environmental matrices. Thanks to these advances numerous monitoring studies have been conducted lately that revealed a wide range of pharmaceutical active compounds (PhACs) present in low amounts but in great diversity in water bodies [1–4].

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310902962502
http://www.informaworld.com

In modern society, an ever increasing number of pharmaceuticals are used for the treatment and prevention of various diseases. Ingested drugs are only partially absorbed by the body. In addition to their increasingly high global consumption rates, this results in a wide variety of pharmaceuticals along with their metabolites being continuously introduced in domestic wastewaters. Studies have shown that PhACs are only partially removed by conventional wastewater treatment processes used in wastewater treatment plants (WWTPs) resulting in the discharge of contaminated effluent into the receiving water bodies [1,5,6].

β-blockers like atenolol are used in the treatment of high blood pressure as well as in recovery from heart attacks. In several studies, traces of these substances were detected in wastewaters, as well as in surface and groundwaters, indicating their incomplete removal in WWTPs [3,5–8]. Despite the low concentrations detected, those studies also show some damaging effects of these compounds on aquatic ecosystems [6,8].

With the aim of improving the efficiency of WWTPs in removing PhACs such as atenolol and others, optimisation of wastewater treatment processes has been attempted, e.g. by increasing sludge residence times. Some advanced technologies have also been evaluated such as advanced oxidative processes, activated carbon adsorption, membrane filtration and membrane bioreactors [1,9–13]. However, despite the sometimes high removal efficiencies attained, these processes are generally not cost-effective on a large scale [1]. Consequently, there is a growing need for new wastewater treatment systems for removing PhACs from waters that have higher efficiencies at reasonable costs of operation/maintenance.

Subsurface flow constructed wetland (SSFCW) systems are low cost wastewater treatment systems consisting of inundated vegetated beds, designed to emulate the well-known water depurative capacity of natural wetlands [14]. These systems are increasingly being used to provide a form of secondary or tertiary treatment for wastewaters. They have already been used with success to remove some organic recalcitrant compounds from contaminated waters such as pesticides, PAHs, organic solvents or explosives [15–17]. However, only a few studies have until now been conducted on the removal of pharmaceutical residues [18–23] probably due to the only recent awareness and concern with these water contaminants.

Wastewater depuration in SSFCWs is achieved by the concerted action between plant rhizomes, microorganisms and the support matrix components. SSFCWs' efficiency can be significantly improved by optimisation of the operation conditions. The performance of these systems is highly dependent on the solid matrix materials and the plant species chosen. When designing a SSFCW it is important to select a matrix with a high sorption capacity, which will depend on the physico-chemical properties of the material chosen. Previous studies have shown that light expanded clay aggregates (LECA) are able to remove, by sorption, other pharmaceuticals from water [23,24]. With respect to the vegetation, aquatic plants play a central role in the depuration mechanisms occurring in a SSFCW. They provide support for the growth of microorganisms and promote the removal of a variety of pollutants by adsorption on the roots, uptake or degradation [14,25]. The most commonly used emergent vegetation in SSFCWs includes macrophyte species such as the cattail (*Typha* spp.) and the common reed (*Phragmites australis*) [14,26,27].

The aim of the present work was to evaluate the efficiency of microcosm constructed wetland systems to remove atenolol from contaminated wastewater, using LECA as the solid matrix, and planted with *Phragmites australis* or *Typha* spp. plants.

For pre-concentration of the samples, a SPE (solid-phase extraction) method was developed and optimised using water and wastewater spiked with atenolol which was quantified using high performance liquid chromatography coupled with a diode array detector (HPLC-DAD) instrumentation.

2. Experimental

2.1 *Atenolol removal by microcosm SSFCW systems*

2.1.1 *SSFCW microcosm assays setup*

Nine SSFCW microcosms were built using PVC containers (0.6 m long × 0.5 m wide × 0.4 m deep) filled with washed LECA (2/4) with a depth of 0.3 m. Water level was maintained just below the LECA surface, corresponding to a flooding rate of approximately 100%. Three beds were planted with pre-grown reeds (density of 20 plants/m^2), three beds were planted with pre-grown cattails (density of 20 plants/m^2) and another three were left unplanted. In addition to the nine SSFCW microcosms, three additional vessels were used, filled only with the tested wastewater solutions and without any plants or solid media, for the purpose of assessing the biodegradation of atenolol in the wastewater alone (see Figure 1).

2.1.2 *Physical and chemical characterisation of the support matrix*

In this study, LECA with a granulometric grade of 2/4 (commercial name Filtralite® NR 2-4), that was used as the solid matrix for the SSFCW microcosms, was supplied by MaxitGroup Portugal.

The commercially available media contain considerable amounts of fine materials which were significantly reduced by washing the LECA material with Millipore water (Simplicity® UV, Millipore Corp.) until no further suspended materials were visible. The washed media were then air dried and used throughout this study.

The particle-size distribution on a weight basis was analysed in triplicate by the conventional dry-sieving technique [28]. Grain-size distribution plots were used to estimate d_{10} (effective grain size) and d_{60}, and the uniformity coefficient ($U = d_{60}/d_{10}$). The apparent porosity (void space) of the media was determined from the amount of water needed to

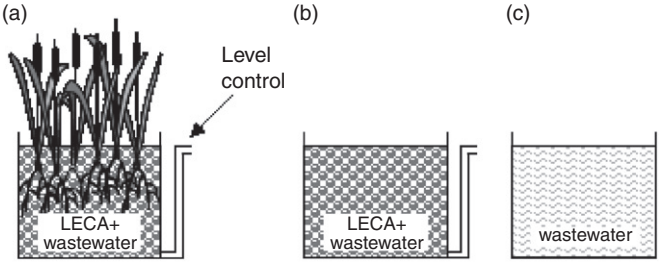


Figure 1. Schematic diagram of the experimental setup used in the atenolol removal assays; (a) the planted beds (with either *Phragmites* or *Typha*), (b) unplanted LECA beds and (c) the wastewater only systems. Three replicates of each system were constructed.

saturate a known volume of the solid [29,30]. Bulk density was determined based on the ratio between the dry weight and the bulk volume of the media [29]. Hydraulic conductivity was measured as described by Cooper [27]. All these measurements were replicated five times.

The mineralogical composition of the media was studied by X-ray diffraction (XRD) using a Bruker AXS-D8 Advance diffractometer with Cu Kα radiation and a speed of 0.05 °/s, from 3 to 75°: 2θ, after grinding the samples so as to pass a 106 µm sieve.

2.1.3 *Plant collection and acclimation*

Phragmites australis and *Typha* spp. rhizomes with shoots were collected in water streams in Alentejo, Portugal, during April 2007. The rhizomes were thoroughly washed to remove any soil/sediment particles attached to the plant surfaces and then were placed in vessels for acclimation. An aerated modified Hoagland nutrient solution was used, adapted from Fediuc and Erdei [31], having the following starting chemical composition, with pH adjusted to 6.0 : 2.5 mmol L^{-1} K^+, 2 mmol L^{-1} Mg^{2+}, 2 mmol L^{-1} Ca^{2+}, 2 mmol L^{-1} SO_4^{2-}, 6 mmol L^{-1} NO_3^-, 0.5 mmol L^{-1} $H_2PO_4^-$, 10 µmol L^{-1} Fe^{3+}, 10 µmol L^{-1} H_3BO_3, 1 µmol L^{-1} Mn^{2+}, 0.5 µmol L^{-1} Cu^{2+}, 0.1 µmol L^{-1} MoO_4^{2-}. In the case of *Typha* this solution was used without any dilution, but in the case of *Phragmites* better shoot development was obtained with a dilution to 10% of the starting Hoagland solution. The nutrient solution was replaced twice every week.

Plants were grown in a growth chamber (Fitoclima, Portugal) at 22°C, with 70% of relative humidity and a light-dark cycle of 12 : 12 h. The photon flux density was 270 µmol $m^{-2}s^{-1}$. After 6 weeks, when new roots and leaves had developed, plants of uniform size were selected and planted in LECA beds, in a greenhouse, where they were set for a period of acclimation of approximately one year.

2.1.4 *SSFCW microcosms operation and sample collection*

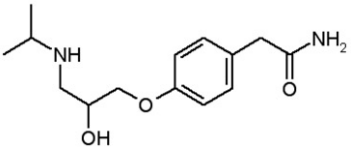
Both the SSFCW microcosms as well as the three additional empty vessels described in (2.1.1) were filled with wastewater spiked with atenolol at 0.78 µg mL^{-1}. This solution was prepared from a wastewater collected at a secondary treatment stage in a WWTP serving a small rural community population of *ca.* 400 inhabitants. The treatment processes used in this WWTP include screening, primary sedimentation and conventional activated sludge treatment. The wastewater collected at the WWTP was spiked with atenolol by dissolution of a stock aqueous solution of 100 µg mL^{-1} of atenolol.

The systems were operated in a batch mode, i.e. with the initial load of the solution and without any solution flow during the assay.

During the assays, samples of atenolol solution were collected after the periods of 6, 12 and 24 hours for the first day and then with a daily periodicity for a total of 8 days of retention time. Collection of the liquid was made at half depth of the beds at random points on the beds surface. After collection, the samples were kept refrigerated until the time of analysis, which was always carried out within 2 hours from the time of collection.

Evapotranspiration in the beds was daily controlled and the water volumes lost through evapotranspiration were restored with distilled water.

Table 1. Relevant physical-chemical properties of atenolol [5].

Common name	Atenolol
IUPAC name	2-[4-[2-hydroxy-3-(1-methylethylamino) propoxy]phenyl]ethanamide OR 2-(4-(2-hydroxy-3-(isopropylamino) propoxy)phenyl)acetamide
CAS number	29122-68-7
Pharmacological class	β-Blocker
	90% excreted unchanged
Structure	
Molecular weight ($g\,mol^{-1}$)	266.34
Melting point (°C)	152.0
Ionisation constant, pKa	9.6
Water solubility (25°C) ($mg\,L^{-1}$)	13,300
Log Kow	0.16

2.2 *Reagents and materials*

Atenolol (≥98% purity) was purchased from Sigma-Aldrich (Steinheim, Germany). Some of the most relevant physical-chemical properties of this pharmaceutical are listed in Table 1. HPLC gradient grade acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). All other high purity chemicals and solvents were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and Panreac Quimica SA (Barcelona, Spain), and were used without further purification. Ultra-pure water was obtained with a Milli-Q water purification system (Simplicity® UV, Millipore Corp., France).

The cartridges used for solid phase extraction were: LiChrolut® C_{18} (500 mg, 3 mL) from Merck (Darmstadt, Germany), and Sep-Pak® Vac (500 mg, 3 mL) and Oasis® HLB (200 mg, 6 mL) from Waters Corporation (Milford, MA, USA). Filters with 0.45 μm nylon membrane were purchased from VWR International (West Chester, PA, USA).

2.3 *Analytical methods*

2.3.1 *Wastewater characterisation*

The collected WWTP effluent was characterised by the determination of the following wastewater quality parameters, according to the APHA-AWWA-WPCF methods [32]: total suspended solids (TSS), pH and total and soluble chemical oxygen demand (COD_t and COD_s) of samples filtered through 0.45 μm filters.

2.3.2 *Solid phase extraction*

Several SPE cartridges were tested with spiked water and wastewater for the extraction of atenolol: LiChrolut® C_{18}, Sep-Pak® Vac and Oasis® HLB (conditioned with 10.0 mL of

methanol and 10.0 mL of water). All columns were tested at two different sample pH conditions, namely pH = 12 (adjusted with NaOH) and without any pH adjustment (pH ~ 7–8). Three replicates were done for every test.

After sample filtration through 0.45 μm filters, the samples were percolated through the cartridges. Afterwards some cartridges were rinsed with 5.0 mL of Milli-Q water to test the influence of a washing step. The cartridges were then air dried for about 15 min under vacuum to remove excess water. The analyte (atenolol) retained in the columns was eluted with 5.0 mL of methanol. Following elution, the solutions were evaporated on a rotary evaporator at 30°C to dryness and redissolved with 1.0 mL of Milli-Q water.

The optimised SPE conditions used for the analysis of the remaining atenolol in the SSFCW microcosm assays were as follows: the samples were filtered through 0.45 μm filters and their pH adjusted to 12 before being percolated through LiChrolut® C_{18}. The cartridges were then rinsed with 5.0 mL of Milli-Q water and dried under vacuum conditions for 15 min after which the atenolol was eluted with 5.0 mL of methanol. Solutions were evaporated on a rotary evaporator at 30°C to dryness and redissolved with 1.0 mL of Milli-Q water. Three replicate analyses were done for each plant assay.

2.3.3 *Quantification of atenolol and analytical method validation*

Analysis was performed using HPLC equipment Agilent 1100 with a DAD detector (Agilent Technologies, Germany). The reversed phase analytical column used was a Zorbax Eclipse XDB-C_8 (4.6 mm × 150 mm) with 5 μm particle size. The DAD detector was scanned from 200 to 500 nm, and the chromatographic profile was recorded at 230 nm.

The separation was performed in isocratic mode, and the mobile phase used was composed of 10 : 90 (v/v) acetonitrile : water, at a flow rate of 1.0 mL min^{-1}. The water was acidified with 0.1% (v/v) phosphoric acid. All analyses were performed at room temperature and the injection volume was 20 μL. Three replicate injections were made for each sample previously filtered through a 0.45 μm filter.

Identification of the atenolol peak in the HPLC-DAD chromatogram was achieved by comparing the retention time and UV spectra of each sample with that of the corresponding atenolol reference and, whenever necessary, co-elution studies were performed. Calibration curves were constructed using a standard solution of 100.0 μg mL^{-1} of atenolol to prepare the standards of 0.20, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0 and 5.0 μg mL^{-1}. Three replicates were made for each standard solution, and each solution was injected five times. Instrumental quantification and detection limits (IQL and IDL) for the chromatographic measurement were determined as the analyte concentrations giving a signal equal to the blank signal plus 10 standard deviations, and plus three standard deviations, respectively [33]. The repeatability of the HPLC-DAD system was tested by performing six consecutive replicate injections of the same standard solution using the same mobile phase, and it was evaluated as the dispersion (relative standard deviation) of the measured peak areas. The reproducibility of the HPLC-DAD system was determined by performing injections of six different standard solutions in different days always using fresh solvent as the mobile phase each day, and it was evaluated as the dispersion (relative standard deviation) of the measured peak areas.

In order to test for possible negative effects on the LiChrolut® C_{18} column's performance due to the use of large sample volumes, a series of trials were also performed using varying volumes of solution (5, 50 and 100 mL) all containing the same amount of the analyte. The effect of atenolol concentration was also investigated using wastewater

samples spiked with atenolol at concentration levels of 0.5, 1.5 and 2.5 $\mu g\,mL^{-1}$, for the same volume (5.0 mL) of sample percolated through the SPE column. Three replicate analyses were performed for each sample volume percolated and atenolol concentration level. Atenolol absolute recoveries were calculated as the ratio of the peak areas obtained by HPLC-DAD in the solid phase extracted sample and in the non-extracted standard. The average absolute recovery percentage obtained for the different volume assays was used to calculate the analytical method's LOQ.

Limits of quantification of the entire analytical methods (LOQ) were calculated following the equation [34]:

$$LOQ = (IQL \times 100)/(Rec(\%) \times C)$$

where IQL is the instrumental quantification limit ($\mu g\,mL^{-1}$), Rec (%) is the average absolute recovery of atenolol in wastewater samples and C is the concentration factor (100).

The reproducibility of the entire analytical method was determined by performing, in different days, quantification of atenolol recovered from five spiked wastewater solutions with different atenolol concentrations (0.5–2.5 $\mu g\,mL^{-1}$) and sample volumes (5–100 mL), always using fresh solvent as the mobile phase each day. Reproducibility was evaluated as the dispersion (relative standard deviation) of atenolol recoveries.

3. Results and discussion

3.1 *Analytical method for the quantification of atenolol*

3.1.1 *Chromatographic analysis*

The quantification of pharmaceuticals such as atenolol is mainly performed using chromatographic techniques like liquid chromatography coupled with a mass spectrometer using electrospray ionisation (LC-ESI-MS), LC-ESI-MS tandem and gas chromatography coupled with a mass spectrometer (GC-MS) because of the lower quantification limits that these techniques can achieve ($ng\,L^{-1}$ range) [35]. However, the ESI-MS detector has a significant drawback associated with the ESI ionisation which is highly susceptible to other components in the matrix that may induce signal suppression (more often) or signal enhancement leading to erroneous results [5]. The determination of pharmaceuticals in water samples using GC-MS always requires sample derivatisation. In the β-blockers case, a two-step derivatisation by silylation of the hydroxy groups and trifluoroacetylation of the secondary amino moieties is needed. Since the derivatisation of the hydroxy groups can be incomplete, the use of GC-MS can become inappropriate for the quantification of those pharmaceuticals in water samples [35]. HPLC-DAD, when coupled with an appropriate method of analyte concentration, can be a suitable and less expensive alternative for the determination of trace organics such as atenolol. In this work, an HPLC-DAD method was developed to evaluate the efficiency of SSFCW microcosms to remove atenolol from contaminated wastewater. To optimise the chromatographic separation, a series of preliminary experiments were performed, testing different mobile phases consisting of methanol, acetonitrile or mixtures of methanol and acetonitrile as organic solvent and water with different additives, such as formic acid and phosphoric acid. The best quantification conditions were achieved using isocratic separation with the mobile phase composed by 10 : 90 (v/v) acetonitrile : water acidified with 0.1% (v/v) phosphoric acid. The linearity range ($R^2 > 0.99$) determined for the atenolol standards,

Table 2. Method validation parameters.

	LC-UV system				Entire analytical method	
Linearity range ($\mu g\ mL^{-1}$) (R^2)	IQL ($\mu g\,mL^{-1}$)	IDL ($\mu g\,mL^{-1}$)	Repeatability % RSD	Reproducibility % RSD	LOQ ($\mu g\,mL^{-1}$)	Reproducibility % RSD
0.2–60 (0.991)	0.81	0.24	1.52	2.51	0.009	3.69

Notes: IDL and IQL: instrumental detection and quantification limits; LOQ: limit of quantification of the entire method.

the IDL and IQL of the chromatographic separations calculated according to Miller and Miller [33], the repeatability, the reproducibility and the LOQ of the analytical method developed for the HPLC-DAD equipment, calculated according to Vieno *et al.* [34], are presented in Table 2.

3.1.2 *SPE method optimisation*

In order to optimise a solid-phase extraction method for the pre-concentration of atenolol in wastewater samples, several SPE columns were tested, including one polymeric sorbent (Oasis HLB®) and two apolar cartridges (Merck LiChrolut® C_{18} and Waters Sep Pak® C_{18}). The performance of the different SPE columns was compared first using water spiked with atenolol at a concentration level of $0.5\,\mu g\,mL^{-1}$. Atenolol is a basic pharmaceutical compound (pKa = 9.6) [5] and at basic pH values, it should be predominantly at the non protonated form, increasing its affinity to the SPE sorbent. In 2006, Gros *et al.* [5] tested several SPE columns to pre-concentrate a number of pharmaceutical compounds, including atenolol, and they concluded that at lower pH values (pH = 2) the recoveries of atenolol from an Oasis HLB® column were significantly lower than those obtained if no pH adjustment was done (neutral pH). However, in that work, the recoveries at more basic pH values of the sample were not evaluated. In our work, the efficiency of recovery of the different SPE columns was compared at pH 12 and without pH adjustment of the samples (pH about 7). There are several references [5,36] suggesting that a washing step with water, after the percolation of the sample through the cartridge, can clean up the matrix of some interfering compounds and improve the recoveries of the pharmaceuticals in SPE pre-concentration methods. This parameter was evaluated as well. The results obtained with the four different SPE methods tested are shown in Figure 2.

There was no improvement in the SPE recoveries when the sample pH was adjusted to 12. However, when an additional washing step was added to the procedure a significant improvement was observed in the atenolol recoveries, only on the samples where the pH had been previously adjusted to 12. For the optimal conditions, the three tested columns did not show significant differences in terms of atenolol recovery. Therefore, Merck Lichrolut® C_{18} columns were chosen to be used throughout this study because they were less expensive.

SPE method validation was performed using the optimised conditions (sample at pH 12 and using a washing step with 5 mL of water) and wastewater samples spiked with atenolol. In order to test the possible detrimental effect on the atenolol recoveries when large volumes of wastewater were percolated through the LiChrolut® C_{18}, a series of SPE experiments were established, varying the sample volumes but maintaining the same

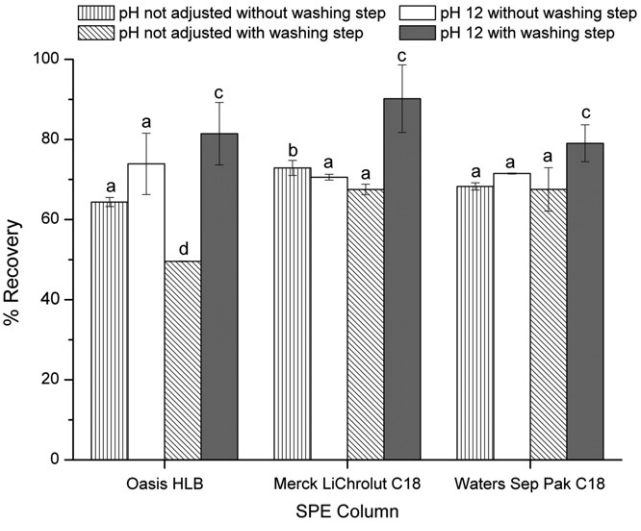


Figure 2. Influence of different SPE materials, pH adjustment (7 and 12), and wash step on the atenolol recovery from water samples. Vertical error bars indicate ± SD ($n = 3$). ANOVA significant at $P < 0.05$ when compared with control. Different letters indicate significantly different values.

Table 3. Influence of sample volume and concentration on the atenolol recoveries from spiked wastewater (average ± SD, $n = 3$) obtained using LiChrolut® C_{18} cartridges. ANOVA significant at $P < 0.05$ when compared with control.

	% Recovery		
Atenolol concentration ($\mu g\,mL^{-1}$)	0.5	1.5	2.5
	83.85 ± 2.33	84.77 ± 1.93	85.59 ± 3.91
Volume (mL)	5	50	100
	85.59 ± 4.01	85.58 ± 3.75	83.26 ± 1.36

atenolol amount. Data presented in Table 3 prove that increasing volumes by a factor of 100 has negligible influence in the recoveries of atenolol. Additionally, higher concentrations of atenolol in the samples (up to 2.5 $\mu g\,mL^{-1}$) did not seem to decrease the recoveries of the SPE method.

Overall the low quantification limit of the entire analytical method (calculated according to Vieno [34] and found to be 9 $ng\,mL^{-1}$, see Table 2), along with the high reproducibility of the analytical method (RSD < 4%, see Table 2) proved that the developed analytical method was suitable to be used in the determination of atenolol in wastewater.

3.2 *Atenolol removal by SSFCW microcosms*

3.2.1 *Physical and chemical characterisation of solid matrix and wastewater*

LECA (2/4) used in the assays was quite uniform in terms of particle size ($U = 1.38$) with most of its particles (95%) having diameters within 2.83–5.00 mm. LECA presented

a pronounced alkalinity (pH in water of 9.01 ± 0.02 and PZC of 9.67 ± 0.03). These characteristics may be attributed to the presence of alkaline components such as metal oxides and carbonates, as was verified in a media mineralogical characterisation by X-ray diffraction (data not shown). The apparent porosity (or void space) of LECA is quite large ($48\% \pm 1$), which may contribute to the good hydraulic conductivity measured ($7.7 \times 10^{-3}\,m^3\,m^{-2}\,s^{-1}$).

The wastewater used in the assays was collected after a secondary treatment stage in a WWTP of a small rural community. Some of the most common parameters used to characterise wastewater quality were evaluated and are presented in Table 4. Organic load and suspended solids for this wastewater were, at the time of collection, somewhat high but still within the Portuguese legal limits for discharge into water streams.

Table 4. Physical and chemical properties ($\pm$SD, $n = 5$) of the treated wastewater used in the assays.

Parameters	Treated wastewater
pH	8.06 ± 0.05
TSS ($mg\,L^{-1}$)	47 ± 3
COD_t ($mg\,L^{-1}$)	127 ± 2
COD_s ($mg\,L^{-1}$)	76 ± 2

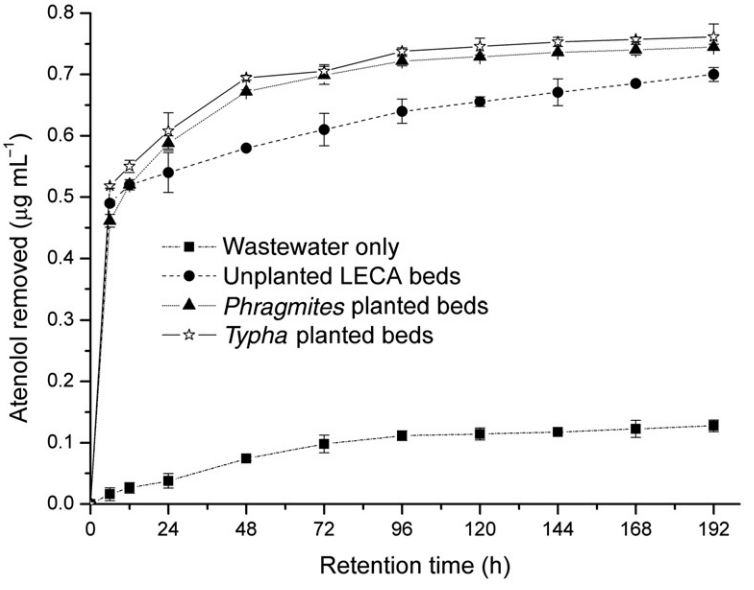


Figure 3. Kinetics of atenolol removal by the *Phragmites* and *Typha* planted beds as well as the unplanted LECA beds. Also depicted is the kinetics of atenolol biodegradation in the wastewater only. Vertical error bars indicate $\pm$ SD ($n = 3$).

3.2.2 *Kinetics of atenolol removal*

The effect of contact time and type of vegetation (*Phragmites* or *Typha*) in the removal of atenolol from spiked wastewater is shown in Figure 3 and compared with unplanted LECA beds. In the same figure, the kinetics of the biodegradation of atenolol in the wastewater (i.e. not in contact with planted or LECA beds) is also presented.

In every assay the kinetics are characterised by an initial fast step (first 6 hours) that is mostly due to adsorption over the LECA's surface (although, in the planted beds, some adsorption onto the plant's roots is likely to occur also) through which more than half the initial atenolol is removed within this short period. Subsequently, a slower process is responsible for additional compound removal. At this stage, removal in the unplanted LECA beds is slower than in planted ones. After 96 hours, almost no further atenolol is removed in planted beds while it continues to be removed in unplanted LECA beds until the end of the assays. However, the amounts of atenolol removed in the planted beds at 96 hours are higher than those removed in the unplanted LECA beds at 192 hours (see Figure 3).

The kinetic behaviour observed for the sorption process occurring in the unplanted LECA beds has similarities with that observed for other compounds sorbed on the same material, namely other PhACs such as clofibric acid, ibuprofen and carbamazepine [23,24]. After the first step of 6 hours, the process has been observed, in the period of 6 to 96 hours, to follow a first-order kinetics which fits the equation

$$\ln[\text{atenolol}](t) = -1.23 - 0.0077\,\text{h}^{-1}t \quad R^2 = 0.994 \tag{1}$$

In first-order kinetics the half-lives of the consumed species are independent of their initial concentrations for a given removal rate constant. For this process, therefore, a similar profile should be followed by the time evolution profile of relative concentrations removed ($[\text{PhAC}]_t/[\text{PhAC}]_0$) for all the initial concentrations of the compound.

Considering the profile of the atenolol removal kinetics, very little advantage can be obtained in terms of percent removal by extending beyond 96 hours the retention time of the atenolol spiked wastewater in the planted systems.

3.2.3 *Atenolol removal efficiency by the SSFCW microcosms*

Within the period up to 96 hours, as much as 93% and 95% of atenolol were removed by the *Phragmites* systems and the *Typha* systems, respectively. A decrease in atenolol concentration is also observed (Table 5) in the wastewater only, not submitted to any treatment, which may be indicative of the atenolol biodegradability by the microorganisms

Table 5. Removal efficiencies ± 1 SD ($n = 3$) of atenolol in SSFCW microcosm assays as well as in the unplanted LECA beds and in the wastewater only, after 96 h of retention time.

System	Atenolol removed (%)
Wastewater only	14.3 ± 1.2
Unplanted LECA beds	82.0 ± 1.5
Phragmites planted beds	92.5 ± 0.6
Typha planted beds	94.5 ± 1.1

present in the wastewater. As can be observed, the biodegradability of atenolol is rather low, as has already been reported in other studies [1,4,6].

For the same period of 96 hours, the unplanted LECA beds were able to achieve high removal efficiencies (82%, Table 5) that are indicative of the strong sorption capacity of this material towards atenolol. The good sorbent characteristics of LECA have already been reported for other pharmaceuticals such as the acidic compounds clofibric acid and ibuprofen as well as the neutral carbamazepine for which the sorption by LECA is also high [24]. Electrostatic interactions for the case of the acidic pharmaceuticals and van der Waals interactions for the case of the neutral compounds have been hypothesised as being responsible for the affinities of these compounds towards LECA's surface. However, considering that both LECA and atenolol have alkaline nature and both are positively charged at working pH conditions (pH $\sim$ 8) the influence of electrostatic interactions does not explain the strong sorption of atenolol onto LECA. Probably, for this compound, ion exchange phenomena may be responsible to some extent for the efficiency of atenolol removal by LECA. This mechanism is known to be responsible for the removal into clay materials of other charged organic compounds such as some pesticides [37–39].

Enhanced efficiency is achieved by the planted LECA beds in comparison with that observed for the unplanted beds (Table 5). Even though most atenolol is retained at the solid matrix, the presence of the plants, either *Typha* or *Phragmites*, contributes with additional 12–14% efficiency in comparison with that due to the LECA material alone. This contribution by the plants is consistent with the contributions reported by other authors [14]. In addition to other benefits for the SSFCW system operation which derive from the presence of plants [14,25,27], these results demonstrate the equally important and active role played by the vegetation in the removal of atenolol, which enables the performance of planted systems to surpass that of a simpler LECA filter setup.

The comparison between the performance attained with *Phragmites* and *Typha* planted beds seems to suggest that the latter species is somewhat more efficient than the former one. However, it should be noted that although both *Typha* and *Phragmites* plants were planted in the beds at the same time, it was observed (by visual inspection) that during the acclimation period aerial parts of *Typha* were slightly more grown than were those of *Phragmites*. Also, probably due to a more developed aerial part, the transpiration rate of *Typha* was higher than for *Phragmites* (data not shown) which may have contributed to the slightly higher efficiency of the *Typha*.

The overall performance of these SSFCW systems, either planted with *Typha* or *Phragmites*, is largely superior to that reported for atenolol removal by the conventional processes used in WWTP [1,3,4]. This type of system is being increasingly used as an alternative form of tertiary treatment stage in WWTPs and the present results suggest that it may be an efficient and cost-effective solution to deal with contamination of wastewaters by pharmaceuticals.

4. Conclusion

In the present study, two types of LECA-based SSFCW microcosm planted with two different macrophyte species, *Typha* and *Phragmites*, were tested for their atenolol removal capabilities from wastewaters. The study provides an assessment of the potential of these systems to deal with pharmaceutical contamination.

A chromatographic method of analysis was established to measure atenolol in wastewater. Combined with an efficient SPE concentration step, the use of the widely available and inexpensive DAD detector yielded an analytical method with very low LOQ ($9\,ng\,mL^{-1}$) and high reproducibility (RSD < 4%).

The material LECA, used as solid matrix in the tested microcosms, was responsible for most of atenolol removal from the wastewater, but both plant species used did contribute with an additional 12–14% to the overall removal by the planted systems. In addition, plants significantly increased the rate of atenolol removal, which enables retention time of the wastewater in the planted beds to be reduced to 96 hours in comparison to the much longer times needed to attain the same efficiency in a LECA filter bed. Although *Typha* presented a slightly higher efficiency than *Phragmites*, this may be related to the more advanced stage of development of the former species at the beginning of the assays.

Further tests are still required for assessing the impact on performance caused by more realistic conditions such as the removal during a longer time period with several cycles of loading in a microcosm wetland. Differences in changing from a discontinuous feed (batch mode), such as is used in this work, over to a continuous mode of feed and also a possible decrease in performance when going to a full scale wetland should also be evaluated. Nevertheless, the studied microcosms showed the high efficiency of these systems in removing atenolol and possibly other similar organic compounds from wastewaters and suggest that larger scale SSFCW systems may be efficient and cost-effective alternatives to other high-cost technologies, such as ozonisation or membrane bioreactors, to be used as a tertiary treatment stage.

Another aspect requiring further research is the potential risk of toxicity for the aquatic ecosystems by the residual concentrations still present in the effluents of the planted or the unplanted beds, after the treatment. In fact, even if large percentage removals are attained that does not ensure non toxic levels of the pharmaceuticals in the treated effluent.

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