

PHARMACEUTICALS REMOVAL FROM URBAN WASTEWATER BY MEANS OF BIOLOGICAL AND PHYSICAL-CHEMICAL TREATMENTS IN FULL SCALE PLANTS

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ABSTRACT

During the last ten years international regulatory bodies paid a special attention to "emerging contaminants" in water; these pollutants are present in water in low or very low concentrations, they are ubiquitous and their effects on human health and environment are, in a wide measure, still unknown. The European Community, in the Water Framework Directive (WFD), refers to various products that are not yet subjected to quality standards in water bodies and it established to minimize negative impacts: personal care products (PCPs), flame retardants (PBDE, TCPP), fuel additives (MTBE), plasticizers (DEHP), hormones and pharmaceutical products (PhC).

This paper presents a study about two pharmaceutical products, Carbamazepine (CBZ) and Oxcarbazepine (OXCZ), and six of their metabolites produced after assumption for therapeutic purpose, namely: Acridone; 2hydroxi-CBZ, 3hydroxi-CBZ; CBZ 10,11 epoxide; 10,11-dihydro-10-dihydroxiCBZ; 10,11-dihydro-10,11-trans-dihydroxiCBZ.

These molecules are studied in wastewater of two areas of the Lombardy (Italy) served by two different wastewater treatment plants: one (city of Pavia) has a traditional scheme, with primary and secondary treatment phases, the other (S. Antonino Ticino, Varese), fed with urban and industrial wastewater, has usual primary and secondary treatment, nitrogen removal and tertiary treatment for a treated water quality improvement; after this plant a part of treated water is released into the first ground water level.

The concentration of these molecules in wastewaters was established in theory (taking into consideration data just like consumption of pharmaceutical products and flow) and data obtained in this way were compared with the results obtained by the analysis of about 80 samples of wastewaters collected in the two treatment plants (analysis with Ultra Performance Liquid Chromatography coupled with tandem mass spectrometry, UPLC-MS/MS).

Even if CBZ and OXCZ are molecules very similar, the results of environmental monitoring show different degradation behaviors. Besides, the biological treatment for the removal of organic material and nitrogen is not always effective for the treatment of such compounds, but a strong oxidation (ozonation) seems more effective.

1 INTRODUCTION

Human activities release in the environment, and in particular in water, a huge amount of pollutants. Wastewater treatment technologies are not always useful for the removal of these compounds, in particular the so called “emerging pollutants”. Pharmaceutical compounds (PhC) are inside this group of compounds. More than 3000 drugs are available in commerce in Europe today; about hundred of these represent the best selling PhC.

The study presented here highlights two of these pharmaceuticals; although the use of these two drugs is limited, the whole quantity distributed in Italy is over 20 t/y and the residues can be analyzed in wastewater and in surface water.

2 MATERIALS AND METHODS

2.1 The selected pharmaceuticals

Our group studied in particular two molecules: Carbamazepine (CBZ) and Oxcarbazepine (OXCZ); the first one is sold from 1960, and both are used for neurogenic pain therapy, epilepsy and bipolar personality disorder.

We put attention to these drugs because: they are for human use only; they are distributed only by National Sanitary Service and so the consumption may be monitored; they are used for chronic disease therapy and so the consumption should be constant in time; usually they are permanently assumed for long lasting time and they are distributed in a significant amount. Besides we could compare our results with those published in a French study (*Leclercq M. et al. 2009*).

To evaluate the theoretical concentration of CBZ and OXCZ we assumed that these drugs are excreted unchanged for 5% (CBZ) and 3% (OXCZ) and in form of metabolites for 95-97%. Using data related to the distribution in each area of these pharmaceuticals and data about plants management we calculated the theoretical concentration of each drug in the influents.

With UPLC-MS/MS we analyzed CBZ and OXCZ and six relatives metabolites in about 80 wastewater samples collected in two treatment plants.

2.2 The studied wastewater treatment plants (WWTP)

The first plant is in Pavia (Italy), managed by the ASM Pavia S.p.a.; it treats the sewage of the city of Pavia and 4 little municipalities around the town. It has a treatment capacity of about 160,000 inhabitants and is fed with urban wastewater. The treatment chain is: mechanical pre-treatment, primary sedimentation, biological oxidation with conventional activated sludge (0,15 kg BOD₅/kgSS·d, hydraulic retention time 4 hours) and secondary sedimentation.

The second plant is in S. Antonino Ticino (Varese, Italy), managed by Prealpi Servizi S.r.l., it treats the sewage of the central-south part of Varese province (27 municipalities), with a treatment capacity of about 300,000 equivalent inhabitants, fed with a mix urban-industrial wastewater. The treatment chain is: mechanical pre-treatment, primary sedimentation, biological denitrification, oxidation-nitrification with conventional activated sludge (0,1 kg BOD₅/kgSS·d, hydraulic retention time 9 hours), secondary sedimentation, sand filtration, ozonation for colour removal, disinfection

with sodium hypochlorite and finally, lagooning; this last phase produces a direct infiltration of about 30% of the treated flow in groundwater.

Near this plant there are two pits for groundwater monitoring, upstream and downstream the infiltrating lagoons.

2.3 Sampling

The 80 samples were collected during dry periods, both in cold and warm season, with automatic samplers, in flow-proportional mode. A volume of about 500 mL of samples were stored in polypropylene bottle at -18°C until the analysis.

2.4 Analytical procedures

The chemical standards for the study and the internal standard (Carbamazepine, ^{13}C , D_2) were supplied by Sigma Aldrich (Munich, D) and Toronto Research Chemical (Toronto, CDN).

Before the analysis, samples were filtered with glass microfiber filter (Whatman International GFC type, Ø125 mm); then they were purified with Solid Phase Extraction using Waters Oasis HLB cartridges (60 mg/3 mL), and finally concentrated in a vacuum centrifuge before injection onto UPLC system.

The chromatographic instrument was an Acquity UPLC system (Waters, Milford, MA, USA) coupled with a triple quadrupole Waters TQD mass spectrometer. The Mass Lynx 4.1 software was used for instrumental control, and for acquisition and processing of the data. An MS/MS detector with an ESI interface in negative ion mode (ESI-) was used for quantitative analysis, with acquisition in multiple ion recording (MRM) mode.

The analytical method was inspired by Leclercq (*Leclercq M. et al. 2009*) taking into account the difference between the UPLC-MS/MS and HPLC-MS.

To carry out the quantitative analysis a calibration curve was constructed by spiking ultrapure water (50 mL) with increasing amounts of each reference standard in the range suggested by Leclercq. In particular, the final added concentrations were: 0, 40, 100, 500, 1000, 2000 ng/L for CBZ, OXCBZ, DIOHCBZ and 10 OHCBZ; 4, 10, 50, 100, 200 ng/L for 2OH CBZ, CBZ epoxide, Acridone, 3OH CBZ. The internal standard (Carbamazepine, ^{13}C , D_2) was added to each sample at a concentration of 200 ng/L. After filtration with glass microfiber filter all samples were further treated as follows: 50 mL of each sample was loaded onto Oasis HLB disposable cartridges previously activated with 3 mL of methanol and 3mL of water. The cartridges were then dried for ten minutes and the analytes and the internal standard were eluted with 5 mL of ethyl acetate.

So the eluates were concentrated with a centrifugal concentrator set at a temperature of 45°C and finally reconstituted with 100 µL of water- methanol solution (50:50); finally samples were mixed vigorously with a vortex mixer for 15 minutes, centrifuged at 3000 rpm for 5 minutes and injected onto UPLC system (3 µL).

UPLC separation was performed on a Aquity UPLC BEH C18 (100 mm x 2.1 mm, 1.7 µm) column kept at 45°C, by gradient elution with a mixture containing variable proportions of 3 mM ammonium formate pH 3 and methanol at a flow rate of 0.464 mL/min.

The gradient program was: from 25% to 40% of methanol in 6.21 min, from 40% to 90% of methanol in 2.56 min, held for 3.34 min to permit the washing of the column; from 90% to 25% of methanol in 0.15 min and held for 1.8 min (column re-

conditioning). The total run time was 11.5 min. In this condition retention times of the analytes were: 4.1 min for DIOH CBZ, 4.6 min for 10OH CBZ, 4.7 min for 2OH CBZ, 4.9 min for CBZ EPOX, 5.4 min for AO, 5.7 min for OX CBZ, 5.9 min for 3OH CBZ and 7.6 min for CBZ and for CBZ C13 D2.

The instrumental parameters of the MS/MS detector were optimized by infusing standard solutions (1 mg/L in methanol) of each analyte. Electrospray was operated in negative ion mode (ESI-) and the ionization source parameters were: capillary voltage 3.0 kV, extractor voltage 3 V, RF lens voltage 1.0 V, source temperature 150°C and desolvation temperature 400°C. Nitrogen was used as the desolvation and cone gas with a flow rate of 800 and 50 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 1.4×10^{-3} mbar. The transitions, cone voltage (CV), energy collision (EC) used for quantification of each compounds in ESI- are indicated in the table I.

Table I. compound names and other parameters

Compound name	Parent (m/z)	Daughter (m/z)	CV	EC
AO	195.93	166.88	66	46
CBZ	237.05	194.10	45	25
CBZ C13 D2	242.00	198.00	35	30
3 OH CBZ and 2OH CBZ	252.98	209.88	42	32
OX CBZ	253.05	179.89	32	52
CBZ EPOX	253.08	236.18	22	15
10OH CBZ	254.99	237.18	18	24

3 RESULTS

3.1 Population observed for the study

The data about population was collected by the record of Italian National Institute of Statistic (ISTAT) and are presented in table II.

Table II. Population and annual variation in the areas of the study.

Province	Year 2006		Year 2006		Year 2006		Year 2006	
	Pop.	%var.	Pop.	%var.	Pop.	%var.	Pop.	%var.
Pavia	515,600	-	521,300	+1.1	531,000	+1.9	539,200	+1.5
Varese	848,600	-	855,400	+0.8	863,900	+1.0	871,400	+0.9

3.2 Distributed pharmaceuticals

For the area of Pavia we have specific data about the two observed pharmaceuticals distributed in 5 municipalities (89,000 inhabitants) served by the sewage treated in the Pavia plant.

For the province of Varese we present the data related to the distribution of these pharmaceuticals in the whole area, from these data we extrapolate, on the basis of the inhabitants of each municipality, the quantities of CBZ and OXCBZ distributed in the area served by the WWTP of S. Antonino Ticino. The data are in table III.

Table III. Quantity of CBZ and Ox-CBZ distributed in the areas of the study

<i>Year</i>	<i>2006</i>	<i>2007</i>	<i>2008</i>	<i>2009</i>
Pavia CBZ [kg/year]	38.2	38.1	39.3	n.a.
[variation %]	-	-0.3%	+3.2%	
Varese CBZ [kg/year]	12.4	13.2	16.8	n.a.
[variation %]	-	+6.4%	27.2%	
Varese Ox-CBZ [kg/year]	470.7	479.1	483.1	482.8
[variation %]	-	+1.7%	+0.8%	-0.1%
Policlinic of Pavia CBZ [kg/year]	n.a.	n.a.	0.87	n.a.
Policlinic of Pavia Ox-CBZ [kg/year]	n.a.	n.a.	0.54	n.a.

For the area of Varese we have the annual data of distribution, for the area of Pavia we have the values for each month.

The data about CBZ, with an annual per capite assumption of $0.44 \div 0.55$ [g/cap·y] for Pavia and Varese respectively, are below the range ($0.61 \div 0.98$) presented in other studies for Europe (Roig B., 2010). The OXCBZ have an assumption of 0.19 [g/cap·y] in both areas, but for this PhC we have not reference of other studies.

The data show that the two pharmaceuticals are used by patients mainly at home. If we consider that these kind of therapies are long lasting, it is not probable a significant incorrect disposal in sewage for unused or expired pharmaceuticals.

3.3 Sewage flows

In table IV we present the average daily data of inflow in the two considered WWTP.

Table IV. Mean inflows in the two considered WWTP [m³/day].

<i>WWTP – year</i>	<i>2006</i>	<i>2007</i>	<i>2008</i>
Pavia	41,800	38,900	37,500
Varese	86,400	83,000	107,200

On the basis of the data presented above for the year 2008 we calculate the concentration at the end of the sewage inflowing in each WWTP (presented in Table V), to compare these ones to the data measured in the samples collected during the monitoring campaign (2009-2010) in the above mentioned plants (presented in Table VI).

WWTP	Carbamazepine		Oxcarbazepine	
Pavia	lowest 95.2	highest 166.0	lowest 23.7	highest 54.2
Varese	lowest 148.2	highest 283.5	lowest 30.6	highest 58.5

<i>WWTP</i>	<i>Carbamazepine</i>		<i>Oxcarbazepine</i>	
Pavia	lowest 236.7	highest 302.5	lowest 11.5	highest 46.7
Varese	lowest 44.8	highest 380.6	lowest 8.8	highest 88.8
A-MEC - Average measured environmental concentration	214.2		28.0	

Table VII. Carbamazepine in WWTP. Incoming and out coming minimum, average and maximum concentrations [ng/L], in various Countries.

[illegible]

(continues)						
Spain - Santos	-	530	3,780	-	580	1,180
Switzerland - Hollander	-	550	-	-	600	-
USA - Roig	-	173	274	-	119	187

The differences are probably due to the main factor affecting the data: the various protocols or customs of medics in therapy prescription.

3.5 Treatment effects

The comparison between input and output in the Pavia WWTP is presented in the Figure 1.

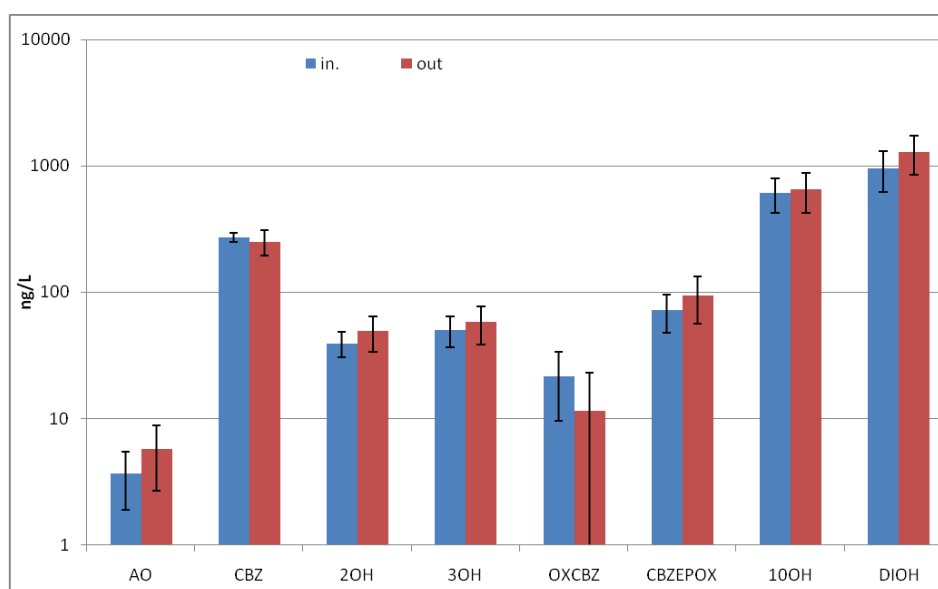


Figure 1. Pavia WWTP. Average values (10 samples) of concentration data [ng/L, **logarithmic scale**] of CBZ, OXCBZ and their metabolites (AO=Acridone, 2OH = 2hydroxi-CBZ, 3OH = 3hydroxi-CBZ, CBZEPOX= CBZ 10,11 epoxide, 10OH = 10,11-dihydro-10-dihydroxiCBZ, DIOH = 10,11-dihydro-10,11-trans-dihydroxiCBZ). in = raw sewage, out = output of treatment. Black line on the bars = standard deviation (SD). In one case (OXCBZ) the SD is greater than the out average value.

In this case of usual biological treatment we can observe no or poor removal of some compounds (CBZ) for many compounds it is clear a growth of average concentration (AO, 2OH, 3OH, CBZEPOX, 10OH, DIOH).

If we consider the behavior of the two commercial PhC, we see that OXCBZ has a better response to biological treatment than CBZ, a molecule considered like a marker of pollution because of its resistance to degradation.

The comparison between the input of PhC and the output from the different treatment phases in the S.A.T. WWTP is presented in Figure 2.

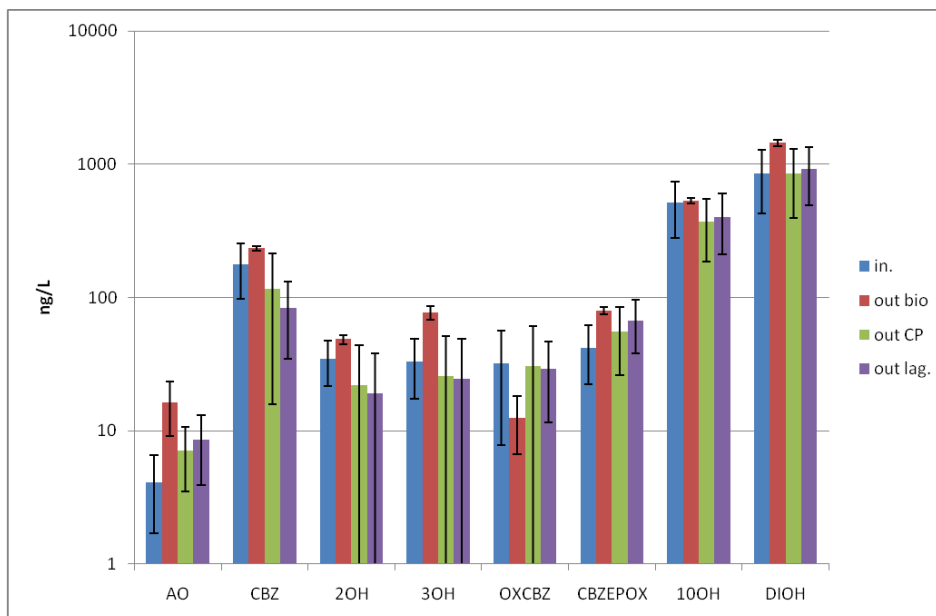


Figure 2. S.A.T. WWTP Average values (21 samples) of concentration data [ng/L, logarithmic scale] of CBZ, OXCBZ and their metabolites (AO=Acridone, 2OH = 2hydroxi-CBZ, 3OH = 3hydroxi-CBZ, CBZEPOX= CBZ 10,11 epoxide, 10OH = 10,11-dihydro-10-dihydroxiCBZ, DIOH = 10,11-dihydro-10,11-trans-dihydroxiCBZ). in = raw sewage, out bio = output of biological treatment, out CP = output of chemical-physical treatment, out lag = output of lagooning. Black line on the bars = standard deviation (SD). In three cases (2OH, 3OH, OXCBZ) the SD for out CP is greater than the average value.

In this case the deeper biological treatment (oxidation-nitrification and pre-denitrification) give a growth of the average concentrations for all compound except the OXCBZ. On the contrary the chemical oxidation, and in particular the ozonation, produces a lowering of the average concentrations for all compound (outgoing from biological treatment) except in the case of OXCBZ. The result of whole treatments is a clear reduction of the average concentrations of CBZ, 2OH, 3OH, 1OH, no effect for OXCBZ and a growth of the average concentrations of AO, CBZEPOX and DIOH.

4 FINAL REMARKS

The analytical methodology developed during this study to find Carbamazepine and Oxcarbazepine in sewage and treated wastewater is rapid, accurate and reliable. It allows determining the presence of pharmaceutical compounds at the concentrations that are usually present in the environment.

The presence of PhC and their metabolites residues in sewage water can be experimentally detected by means of specific chemical determinations, but if we take in account the wide diffusion of PhC in wastewater, we can have a quite forecast of the incoming concentration in wastewater treatment plants on the basis of the distributed quantities of PhC, of its rate of excretion and of sewage flow, without the charge of high analytical costs that are necessary for the determination of each specific molecule.

The usual biological treatments for wastewater are ineffective for the removal of resistant molecules like Carbamazepine. To obtain good removal rates it is necessary to apply stronger treatment like, for example, the ozonation. In WWTP where this treatment is just in use to remove “usual” pollutants (dyes, detergents etc.) we can forecast good removal results also for PhC.

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