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OXIDATION OF PHARMACEUTICALS BY CHLORINE DIOXIDE IN WASTEWATER EFFLUENT

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SUMMARY

The presence of pharmaceuticals in the environment has raised an emerging interest due to the fact that they pose negative environmental impact and health hazards related to long-term toxicity effects. Its removal from wastewater and from important drinking water sources is a big challenge to be faced by wastewater treatment plants (WWTPs) and environmental engineers due to the low concentration that is usually present in the water in addition to its high diversity (chemical structure, mode of action, K_{ow}). Pharmaceuticals end up into the influent of WWTPs mainly through the sewage system that transports excreted faeces and urine from households but they do not have yet maximum discharge guidelines and standards. Conventional treatments used nowadays in WWTPs are not able to totally remove pharmaceuticals from wastewater and therefore it is necessary to seek for alternative advanced technologies such as oxidation with chlorine dioxide (ClO_2). ClO_2 needs to be manufactured at the point of use and it is a highly selective oxidant for several functional groups that are usually present in the chemical structure of pharmaceuticals. The capital cost for a ClO_2 generator is lower than other oxidative technologies such as ozone and therefore it could be a good option for small scale WWTPs (<2000 pe).

Previous studies with ClO_2 have mainly tested the disinfection effect of ClO_2 in both drinking water and wastewater, although during the last years, more research has been conducted in ClO_2 as oxidant for pharmaceuticals focused on how (kinetics) and which pharmaceuticals can be removed. However, the objective of this master thesis was to find the most optimal dose – reaction time of ClO_2 for the maximum removal of selected environmentally relevant pharmaceuticals. This was made as a bench-scale study where factorial design and subsequent optimization with MODDE was selected as the best approach to find the optimal dose – time. Batch oxidation tests were conducted on 100 mL aliquots treated with ClO_2 using wastewater effluent from Henriksdal WWTP. Solid phase extraction and quantification of pharmaceuticals was carried out on a high performance liquid chromatography- triple quadrupole mass spectrometry (HPLC-MS/MS).

At the beginning of the investigation, a priority list of pharmaceuticals was created based on the risk ratio between the concentration at the effluent and the concentration that has no predicted effect in the environment retrieved from Pharmaceutical Specialties in Sweden (FASS) drug portal data base. 3 of the 23 studied pharmaceuticals (oxazepam, metropolol and diclofenac) were classified as high risk, 5 as moderate risk and the remaining as low risk. Results from the bench scale study states that an optimal dose of 5 g ClO_2/m^3 and a reaction time of 10 minutes can totally remove more than a half of the studied pharmaceuticals. Furthermore six of the eight pharmaceuticals that posed moderate and high environmental risk before ClO_2 treatment, would pose a low environmental risk after treatment with ClO_2. Results indicate also that variations in wastewater composition (e.g. COD) would affect the oxidant demand and therefore ClO_2 – sensitive absorbance at 254nm and pharmaceuticals dose-response decay curves were measured as possible strategy for ClO_2 dosage control in full scale WWTPs.

Before a pilot or full scale implementation of this technology can be performed, supplementary and deeper ecotoxicologial studies needs to be done regarding the formation of byproducts after oxidation with ClO_2 that can be more toxic than the parent compound.

SAMMANFATTNING

Läkemedel i miljön har rönt ett växande intresse på grund av att de har negativ miljöpåverkan och utgör en hälsorisk till följd av toxiska effekter på lång sikt. Deras avlägsnande från avloppsvatten och från viktiga dricksvattenkällor är en stor utmaning för reningsverk och miljöingenjörer. Anledningen till detta är de låga koncentrationerna som vanligtvis finns i vattnet samt likheten mellan läkemedlen (olika kemisk struktur, verkningsmekanism, K_{OW}). Läkemedelsubstanser hamnar i inflödet till reningsverk främst genom avloppssystem som transporterar urin och fekalier från hushåll. I dagsläget finns det dock inga riktlinjer och standarder för maximalt utsläpp.

Konventionella behandlingar som används i reningsverk idag kan inte avlägsna läkemedelsubstanser helt från avloppsvatten och därför är det nödvändigt att identifiera avancerade tekniker för uppgradering såsom oxidation med klordioxid (ClO_2). ClO_2 tillverkas vid användningsstället och det är en mycket selektiv oxidant för olika funktionella grupper som vanligtvis förekommer i den kemiska strukturen av läkemedelsubstanser (fenoler, tertiära/sekundära aminer, organiska svavelgrupper). Kapitalkostnaden för ClO_2 är lägre jämfört med andra oxidativa tekniker som ozon och skulle därför vara ett bra alternativ för mindre reningsverk (<2000 pe). Tidigare studier med ClO_2 har främst testat desinfektion effekten i både dricksvatten och avloppsvatten, men under de senaste åren har mer forskning fokuserat på ClO_2 som oxidationsmedel om hur (kinetik) och vilka läkemedelsubstanser som kan tas bort. Syftet med detta examensarbete var dock att hitta den mest optimala dos – reaktionstid av ClO_2 för maximalt avlägsnande av utvalda miljörelevanta läkemedel. Detta utfördes med försök i laboratorieskala studie med faktor försök. För den efterföljande optimering utsågs MODDE som det bästa sättet att hitta den optimala dosen – tid. Oxidations tester genomfördes på 100 mL prover med avloppsvatten från Henriksdal reningsverk som behandlades med ClO_2. Därefter genomfördes fastfasextraktion och kvantifiering av läkemedelsubstanser på en högupplösande vätskekromatografi – trippelkvadrupolmasspektrometeri (HPLC-MS/MS).

I början av studien skapades en prioriteringslista över läkemedel baserad på riskkvot mellan koncentration vid utflödet och koncentration som inte har någon förutspådde effekt i miljön (PNEC). Dessa data hämtades från Pharmaceutical Specialities in Sweden (FASS) databas. 3 av de 23 studerade läkemedel (oxazepam, metoprolol och diklofenak) klassificerades som hög risk ämnen, 5 att utgöra måttlig risk och resterande som låg risk ämnen. Resultat från laboratorieförsöken visade på att en optimal dos av 5 g ClO_2/m^3 och reaktionstid av 10 minuter kan ta bort mer än hälften av de studerade läkemedel. Sex av de åtta läkemedelsubstanser som hade måttlig och hög miljörisk innan ClO_2 behandling skulle medföra en låg miljörisk efter behandling med optimal ClO_2 dos -tid. Resultat visar också att variationer i avloppsvattensammansättning (t.ex. COD) påverkar efterfrågan av oxidationsmedel och därför mättes ClO_2 – känslig absorbans vid 254nm och läkemedels dos-respons kurvor som möjlig strategi för ClO_2 doseringskontroll i fullskala på reningsverk.

Innan ett fullskaligt genomförande av denna teknik kan utföras behövs dock vidare och fördjupade toxikologiska tester om biprodukter eller mellanliggande substanser som kan vara mer toxiska än moderföreningarna.

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Raquel Alcala Borao

TABLE OF CONTENTS

ABBREVIATIONS

API = Atmospheric pressure ionization
CCF = face centered central composite design
COD = Chemical Oxygen demand
ClO_2 =Chlorine dioxide
DBPs = Disinfection by-products
DOC = Dissolved organic matter
E2 = 17 beta-estradiol
EC = Environmental concentration
EC_{50} = Half maximal effective concentration
EDTA = Ethylenediaminetetraacetic acid
EE2 = 17 alpha-ethinylestradiol
FASS = Pharmaceutical Specialties in Sweden drug portal
GAC = granular activated carbon
HAAs = haloacetic acids
HPLC-MS/MS = High performance liquid chromatography coupled to tandem mass spectrometry
IS = Internal Standard
K_{Ow} = octanol/water partition coefficient
LOD = Limit of detection
LOQ = Limit of quantification
NOEC = No observed effect level
NSAIDs = nonsteroidal anti-inflammatory drugs
PAC = powdered activated carbon
PE = population equivalent
PNEC = Predicted no effect concentration
RMS = Response surface modeling
RO = Reverse osmosis
SAC = Spectral absorption coefficient
SPE = Solid phase extraction
THM = Trihalomethane
UVA254nm = Ultraviolet absorbance at 254nm
WWTPs = Wastewater treatment plants

ABSTRACT

The presence of pharmaceuticals in the environment has raised an emerging interest due to the fact that they pose negative environmental impact and health hazards related to long-term toxicity effects. As conventional treatments are not able to totally remove these substances it is necessary to seek for alternative advanced technologies such as oxidation with chlorine dioxide (ClO_2). The objective of this master thesis is thus to find the most optimal dose – reaction time of ClO_2 for the oxidation and maximum removal of selected environmentally relevant pharmaceuticals. Factorial design and subsequent optimization with MODDE was selected as the best approach to find the optimal dose – time. Batch oxidation tests were conducted on 100mL aliquots treated with ClO_2 using wastewater effluent from Henriksdal WWTP. Thereafter solid phase extraction and final determination of pharmaceuticals was carried out on a high performance liquid chromatography- triple quadrupole mass spectrometry (HPLC-MS/MS). Results showed that applying a dose of 5 mg ClO_2/L and a reaction time of 10 minutes, it is possible to remove more than a half of the 17 analyzed substances. Besides most of the pharmaceuticals with high and moderate environmental risk, would pose a low risk for the environment after treatment with the optimal ClO_2 dose – reaction time. Despite the fact that ClO_2 could successfully degrade most environmentally relevant pharmaceuticals, deeper research concerning the formation of toxic by-products after oxidative treatment needs to be done before upscaling this technology to pilot or full scale as a suitable end of pipe technology for pharmaceuticals removal.

Key words: pharmaceuticals; chlorine dioxide; wastewater effluent; environmental risk; factorial design; MODDE

1. INTRODUCTION

In recent years, the existence of micropollutants in the aquatic environment has become an emerging issue because of their recognized negative environmental and human health effects. These micropollutans, also called emerging pollutants, integrate a broad range of both anthropogenic and natural substances such as pharmaceuticals, pesticides, biocides, flame retardants, etc. (Luo et al., 2014). The term "emerging" does not mean that the presence of these substances in the environment is new but it refers to the emerging interest by the scientific community in the presence of these contaminants in the environment as well as the analytical procedures required to their detection (Aga, 2008). Another issue that have raised the interest in these substances is that even though they are usually present in trace concentrations (few ng/l to several μg/l) they may cause negative environmental impact and health hazards related to short-term and long-term toxicity, endocrine disrupting effects and rise of antibiotic-resistant genes in bacteria (Klavarioti et al., 2009). This last fact is very relevant since the increase in antibiotic resistance is a serious threat to our availability to face infection diseases.

1.1. Pharmaceuticals in the environment

The removal of pharmaceuticals from wastewater and from important drinking water sources, such as rivers, lakes or groundwater is a big challenge to be faced by wastewater treatment plants (WWTPs) and environmental engineers due to the low concentration that is usually present in the water in addition to its high diversity. There are several

groups of pharmaceuticals that have been found in the water such as antibiotics, antidepressants, analgesics, anti-inflammatories, anticoagulants, stimulants and antihypertensives among others (Rivera-Utrilla et al., 2013). These drugs are designed in order to be effective at low levels in our bodies and to be resistant against e.g. stomach acid and microbial degradation. The fact that these substances are found in trace concentrations requires the development of more sophisticated and advanced analytical tools in order to accurately determine its concentration (e.g liquid chromatography coupled to tandem mass spectrometry [LC-MS/MS]).

The most relevant characteristics of these pharmaceuticals are: i) large and chemically complex structures with high diversity in shape, structure and molecular weight; ii) some of them are lipophilic and others have certain degree of hydrophilicity. This depends on their octanol/water partition coefficient (Log K_{OW}) since those pharmaceuticals with Log K_{OW} higher than 3 (e.g. diclofenac) tend to be attached to fat matrices and those with Log K_{OW} lower than 3 (e.g. metoprolol) have tendency to be attached to sludge and suspended particles in water (Ejhed et al. 2012) ; iii) pharmaceuticals are polar molecules whose ionization level depends on the pH of the medium; iv) their persistence in the environment differs depending on the pharmaceutical. Some substances (e.g. paracetamol) are degraded through the wastewater treatment process, meanwhile others (e.g. naproxen) can persist for more than one year (Rivera-Utrilla et al., 2013). Actually, those pharmaceuticals with Log K_{ow} values higher than 3 have tendency to bioaccumulate (Ejhed et al. 2012).

According to previous studies about the fate and transport of pharmaceuticals in the environment (Fig.1), pharmaceuticals end up into the influent of WWTPs through the sewage system that transport excreted faeces and urine from households. Therefore, most of the pharmaceuticals come into the aquatic environment through the discharges from WWTPs. Veterinary drugs pose also a considerably risk to contaminate soil and groundwater without previous treatment when liquid manure is utilized as top soil cover. Furthermore the sludge from WWTPs, which may contain pharmaceuticals, may be used as fertilizer in agricultural land leading to a high risk for soil contamination as well as risk of run-off polluting both surface and groundwater resources (Santos et al. 2010).

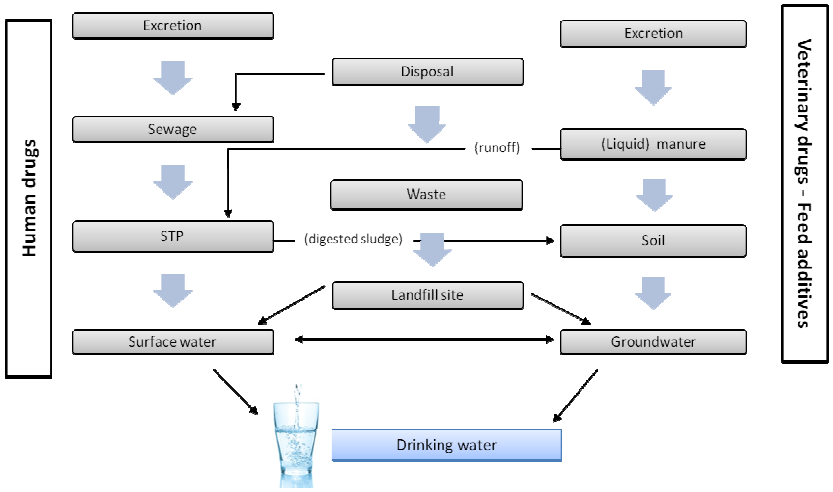

Fig. 1. Fate and transport of pharmaceuticals in the environment (adapted from Ternes, 1998).

Luo et al. (2014) presents in his study a review of average influent and effluent concentrations of pharmaceuticals from different WWTPs around the world where the influent concentration of most of the pharmaceuticals is between 0.1 μg/L and 10 μg/L. The highest concentrations, above 10 μg/L, belong to nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen and naproxen. For instance, concentration levels up to 603 μg/L of ibuprofen were detected in the influent of four WWTPs in Spain (Santos et al. 2010). These NSAIDs can be classified as one of the most relevant group of pharmaceuticals since daily load of these pharmaceuticals to the WWTPs is in the order of grams (Coelho et al. 2010). This is due to the fact that to their application level worldwide is very high, probably because they can be bought without medical prescription.

In Sweden, a large study performed by Stockholm Water, regarding pharmaceuticals in Stockholm´s water environment (Wahlberg et al., 2010) states that NSAIDs such as paracetamol, ibuprofen, naxopren and ketoprofen have the highest concentration in the incoming water of Henriksdal and Bromma wastewater treatment facilities. The highest concentration in incoming wastewater was reached by paracetamol with a value of 85 μg/L.

1.2. Pharmaceuticals and legislation

Discharge of pharmaceuticals is not yet regulated and thus they do not have maximum discharge guidelines and standards. Even though increasing concentrations of pharmaceuticals are entering the environment every day, there is a gap in legislation regarding the environmental contamination produced by them (Luo et al. 2014; Bel et al., 2011; Oller et al. 2011; Santos et al.,2010). This gap in legislation may be due to the absence of consensus and good understanding of which substances should be regulated and to which level. Currently, the World Health Organization says that it is very unlikely that trace concentrations of pharmaceuticals found in drinking water are a risk for human health. This is because the margin of safety between detected concentrations in drinking water and those that can lead to toxic effects is quite substantial (WHO, 2012). However, this current gap in legislation could change if more data concerning long-term exposure is collected and if more knowledge about how a continuous exposure during several generations may affect a whole population is acquired. Besides, information related to the combined or simultaneous exposure to pharmaceuticals is not known yet (Santos et al., 2010).

Recently, the European Parliament has decided for the first time, to include three pharmaceuticals in a 'watch list' of priority substances (Directives 2000/60/EC and 2008/105/EC) in the field of EU Water Framework Directive (European Parliament, 2013). One of these three pharmaceuticals is diclofenac, a commonly-used generic painkiller that belongs to NSAIDs group and that has shown alterations of the kidney and gills of fishes. In some countries such as India, Nepal and Pakistan the manufacture and veterinary use of diclofenac is banned due to imminent extinction of local vultures. The other two substances are sex hormones 17 alpha-ethinylestradiol (EE2) and 17 beta-estradiol (E2), which are suspected to disrupt the endocrine system in humans and harm fish reproduction. By including these substances in the "watch list", monitoring data will be gathered to establish appropriate measures to determine the risk posed by those substances (European Parliament, 2013). Nowadays, the Swedish government together with Swedish Association of Local Authorities has developed a National

Pharmaceutical Strategy that presents seven different action plans of which one of them focus on reducing the impact of medicines on the environment (Läkemedelsverket, 2014).

As there are no specific guidelines for maximum discharge of pharmaceuticals in the environment, the efficiency of WWTPs is usually given as percentage removal between inlet and outlet. However, taking into account possible-ecotoxicological effects in the ecosystem, it would be more interesting to focus on final concentrations at the effluent of WWTPs. One approach would consist on comparing Predicted No Effect Concentration (PNEC) with the environmental concentrations (EC) at the recipient. A dilution factor needs to be considered in order to compare PNEC with EC and if the ratio EC/PNEC is below 1 then long-term negative effects would not be expected.

1.3. Removal of pharmaceuticals in wastewater treatment plants

Current municipal WWTPs can control and eliminate particles, nutrients as P and N as well as reducing the organic load through primary, secondary and optional tertiary treatment process. However they are not meant yet to eliminate micropollutants. Therefore is important to follow the fate of micropollutants in the conventional systems to evaluate the removal efficiency.

1.3.1. Conventional treatments

The main goal of primary treatment is to remove suspended solids from the water through aerated grit chamber followed by sedimentation tank. A study performed by Behera et al. (2011) shows that the maximum removal efficiency was generally quite low (~ 28% for diclofenac and estriol) meanwhile other pharmaceuticals such as ibuprofen and naproxen were not removed at all in this primary treatment. This can be due to the fact that most of the substances have more affinity to be dissociated in water and not bounded to the sludge particles. Actually a study performed by Hörsing et al. (2011) indicates that 61 of the 75 studied pharmaceuticals presented a high affinity to be dissociated in water phase.

In secondary treatment, where substances are transformed by biological degradation through activated sludge and secondary sedimentation, the NSAIDs diclofenac presented low removal (<25%) meanwhile others (ibuprofen, ketoprofen, acetaminphen) presented higher removal above 75% (Salgado et al., 2012; Falås et al., 2012).

The study carried out by Wahlberg et al. (2010) presents a mass balance for 44 different pharmaceuticals performed at Henriksdal WWTP and how they were reduced during the different treatment stages (Fig 2).

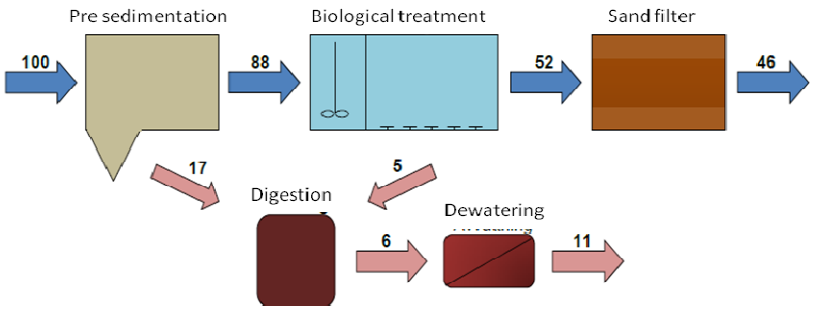

Fig. 2. Mass flow for pharmaceuticals at Henriksdal´s WWTP (adapted from Wahlberg et al., 2010).

The average removal for the 44 pharmaceuticals was around 50% and the highest removal took place in the biological degradation step. Falås et al. (2012) studied the average removal of some pharmaceuticals in activated sludge plants with nitrogen removal where some substances such as ibuprofen and paracetamol where removed by almost 100% (Fig. 3). On the other hand, some pharmaceuticals such as metropolol or hydrochlorothiazide had a negative removal what means that the concentration was higher at the effluent compared to the influent (Fig 3.). The reason of this effect is not totally clear but one possible explanation is that during biological treatment, these substances can be transformed back to parent compounds. In addition to this, some pharmaceuticals may be enclosed in faeces and thus released during biological treatment increasing the effluent concentration levels compared to the influent (Luo et al., 2014).

Consequently, it can be stated that conventional WWTPs are not able to completely remove pharmaceuticals and therefore they are not a complete barrier for them (Vona et al., 2015; Luo et al. 2014; Rivera-Utrilla et al., 2013). Actually only 25% of these persistent substances can be totally removed after secondary treatment, 50% of the substances need additional methods to be removed and 25% shows a negative removal during the process (Hörsing et al., 2014).

Some of the reasons that can explain why pharmaceuticals cannot be effectively removed by biodegradation alone are: i) the low concentration of these substances compared to other pollutants in wastewater may not be enough to active enzymes that are able to eliminate pharmaceuticals; ii) some pharmaceuticals have stable and complex chemical structures that can remain for long time. Besides, many of them are bioactive so they can inhibit growth or metabolism of microorganism and therefore is quite improbable that they can be used as energy or carbon source for microorganisms; iii) the degree of removal will depend on the chemical nature (structure and molecular weight) of the pharmaceutical as well as on the operational conditions of the WWTP (Klavarioti et al., 2009; Aga, 2008). Thus more advanced and specific treatment techniques are needed to increase the removal of pharmaceuticals and decrease the potential impact of the WWTPs effluents in the environment.

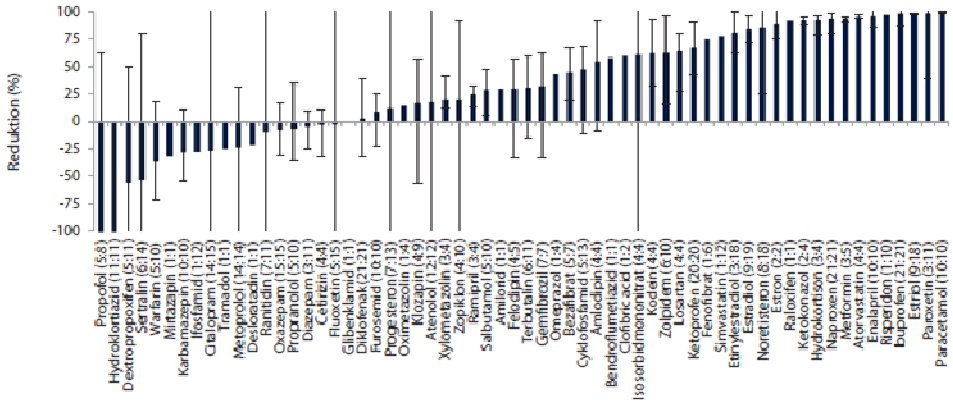

Fig. 3. Average removal and standard deviation in activated sludge plants with nitrogen removal. First number in parentheses indicates the number of facilities where removal was calculated and the second number indicates the total number of facilities that were studied. (Falås et al., 2012).

1.3.2. Advanced treatment alternatives for pharmaceutical removal

The three main options of additional and advanced treatment techniques for pharmaceuticals removal are (Fig. 4): i) improved biological treatment; ii) physical removal by sorption and filtration; iii) advanced oxidation processes.

Improved biological removal can be applied by adding carriers or increasing the sludge retention time. Fålas et al. (2012, 2013) has studied the performance of improved biological removal by using a hybrid moving bed biofilm-active sludge process. In this technique, plastic carriers moves freely inside the bioreactor and at the same time they provide sites for adsorption and growth microorganisms. The result shows that moving bed biofilm carriers can enhance the capacity to remove some compounds such as diclofenac and ketoprofen compared to conventional activated sludge process. Another technique that has been tested for pharmaceuticals removal is membrane bioreactor (MBR) that combines activated sludge biological treatment and membrane filtration. However a rapport carried out by Stockholm Water (Wahlberg et al., 2010) shows that 32 of 46 substances were still detected after treatment with MBR and it gave an average reduction of 80%. Even though these biological treatments has the benefit to have low running cost it seems that this is not a very efficient technique alone to achieve a high level of pharmaceuticals removal.

Physical removal is able to remove pharmaceuticals without degrading them. Membrane techniques such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) use a semipermeable membrane and high differential pressures to remove these substances. However even though MF and UF are efficient to remove turbidity from water, pharmaceuticals are poorly removed because the pores of these membranes are higher than the molecular size of pharmaceuticals. On the other hand, RO presents greater potential to remove pharmaceuticals. A study performed by Yangali-Quintanilla et al. (2011) shows that RO membranes are able to achieve a removal between 85% and 99%.. Even though high removal levels can be reached with this membrane technique there are some important drawbacks. These are related to high electricity consumption and problems with the handling of the rejected flow from the membranes that contains high concentration of micropollutants.

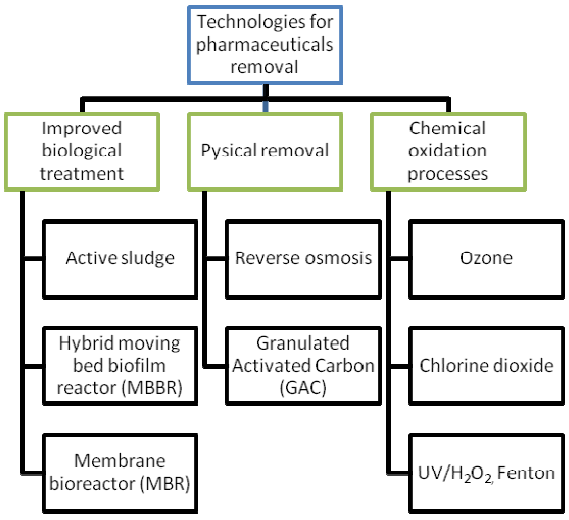

Fig. 4. Some of the available technologies for pharmaceuticals removal.

In order to solve the disadvantages presented by membrane technology, activated carbon adsorption is a technique that can be used to remove persistent/non-biodegradable organic compounds by adsorption processes in the activated carbon pores both with powdered activated carbon (PAC) and granular activated carbon (GAC). In general, efficient removal levels can be achieved with this technology, especially for substances with non-polar characteristics (K_{OW} >2) (Luo et al., 2014). However its efficacy can be affected by the presence of natural organic matter (NOM) that can compete for binding sites, thus leading to blocked pores. One disadvantage for this technology is that large volumes of activated carbon are needed in order to achieve good removal and that there is no disinfecting effect.

To overcome the problems and limitations presented by physical and biological treatment methods, advance oxidation processes are considered. These technologies are based on redox reactions and on the intermediate action of hydroxyl and other radicals that are able to oxidize non-biodegradable substances to several by-products and in some cases to inert end-products (Klavarioti et al.,2008). Besides, chemical oxidants are advantageous because they have disinfecting effect in water. On the other hand, the main disadvantage for oxidation, regardless which oxidizing agent is used, is the formation of new ecotoxicological active compounds that can have undesirable biological effects. These compounds can be either transformation products and/or by-products. Transformation products are those substances that are partially degraded, although the complete mineralization to carbon dioxide and water is not achieved and they can also be substances that are larger than the parent substance after the treatment. These can be less or more toxic substances in comparison to the parent substance (Hörsing et al., 2014). By-products are new substances that are formed during oxidation for instance organo-halogen by-products such as trihalomethanes (THMs) and chlorophenols that are carcinogenic substances formed after treatment with chlorine (WHO, 2004).

The efficiency of these methods will depend on the characteristics of the wastewater (pH, DOC, alkalinity, inorganic substances) as well as how reactive the oxidant is for the target pollutants. The best treatment efficiencies are reached when these oxidation technologies are combined with other physicochemical and biological processes (Klavarioti et al.,2008). For example, if an effluent with biodegradable substances is treated then biological treatment followed by post-chemical oxidation would be the best option. Thus easily biodegradable compounds are first removed and competition for the chemical oxidant is avoided. On the other hand, it may be interesting to have a biological post-treatment after the chemical oxidation in order to remove by biological degradation those by-products that arise from the incomplete mineralization of pharmaceuticals.

There are several oxidizing agents that can be used for this chemical oxidation. A big emphasis has been set on ozone as a promising chemical oxidation technology to remove pharmaceuticals and other organic micropollutants as well as on chlorine dioxide (ClO_2) which is a weaker oxidizing agent compared to ozone but easier to handle. Other advanced oxidation processes such as the combination of ozone with hydrogen peroxide or UV light, Fenton (Fe^{2+}/H_2O_2) or combinations between them have not show results that justify its utilization due to the fact that they are more expensive and complicated to use than ozone or ClO_2 alone (Hörsing et al., 2014)

2. AIM OF THE STUDY

Despite the fact that there are several advanced treatment technologies that can be utilized for pharmaceuticals removal, this study has the objective to investigate and evaluate the effectiveness of ClO_2 as chemical oxidation technology in a tertiary step for the removal of pharmaceuticals in biologically treated wastewater effluent. A bench-scale test study was designed to be able to determine the optimal ClO_2 dose-reaction time relationship based on the maximum level of selected environmentally relevant pharmaceuticals that is possible to remove with this oxidation treatment. Concentration of the oxidant remaining in solution after removal of the pharmaceuticals wants also be investigated and evaluated. Furthermore the possibility of ClO_2 dosage control strategy for future upscaling wants to be studied by evaluating the relationship between ultraviolet absorption at 254nm and pharmaceuticals removal.

3. BACKGROUND

As the current investigation is focused on the application of ClO_2 as oxidative agent to remove pharmaceuticals from biologically treated wastewater, some chemical aspects and previous studies with ClO_2 are described.

3.1. Chemical aspects of chlorine dioxide

ClO_2 is an oxidant that has been widely used as disinfectant of high quality water such as groundwater, treated water and drinking water as well as for wastewater disinfection (Huber et al. 2005). ClO_2 is a neutral compound of chlorine in the +IV oxidation state. When there are not oxidizable substances and in basic medium, it dissolves in water and discomposes into the formation of chlorite and chlorate:

$$2ClO_2 + H_2O \rightarrow ClO_2^- + ClO_3^- + 2H^+ \quad \text{(Eq.1)}$$

ClO_2 is a relatively small, volatile, highly energetic molecule considered as a free radical. Another important physical property is its high solubility in water, especially in chilled water. Actually ClO_2 is 10 times more soluble than chlorine (above 11 ºC) (EPA, 1999)

Concentrated ClO_2 vapor is potentially explosive so it cannot be stored commercially as gas. Therefore it must be manufactured at the point of use. Dilute solutions of ClO_2 must be kept in closed recipient in absence of light since ClO_2 discomposes with sunlight. However an aqueous solution containing >8 g/l of ClO_2 at temperature above 30 ºC is explosive (Hoigné and Bader, 1994). A common reaction to produce aqueous solution of ClO_2 at the point of use is by mixing hydrochloric acid (HCl) and sodium chlorite ($NaClO_2$):

$$5NaClO_2 + 4HCl \rightarrow 4ClO_2 + 5NaCl + 2H_2O \quad \text{(Eq. 2)}$$

ClO_2 is usually preferred to chlorine for disinfection of water. The first reason is because ClO_2 does not produce halogen-substituted disinfection byproducts (DBPs) compounds (e.g. organochlorine and THMs) as chlorine does. This is because ClO_2 reacts as an electron acceptor and H atoms in activated organic C-H or N-H structures are thus not substituted by Cl (Hoigné and Bader, 1994). Besides, chlorine reacts via both oxidation and electrophilic substitution meanwhile ClO_2 reacts only by oxidation. Secondly, ClO_2 is an efficient disinfectant in a broad range of pH between 5 and 10 and it is not affected by the presence of ammonia. Actually ClO_2 is not consumed by ammonia so it is considered a virucide when ammonia is presented in water. Thirdly, ClO_2 does not oxidize bromide. This means that bromide is not

transformed into hypobromite and consequently neither bromoform nor bromated are generated in the water solution (Hoigné and Bader, 1994). Fourthly ClO_2 is more selective in water compared to chlorine and therefore lower disinfectant doses are needed (WHO, 2004).

Accordingly to the study of reaction kinetics of ClO_2 in water performed by Hoigné and Bader (1994), ClO_2 is a highly selective oxidant with several functional groups of organic substances such as phenolic compounds, tertiary/secondary amines as well as organosulfur groups. It is known that many pharmaceuticals have phenolic and/or amino functional groups in their structure therefore it is expected that ClO_2 will oxidize rather high number of pharmaceuticals despite its lower oxidation potential compared to ozone (Huber et al., 2005).

3.2. By-products formation with ClO_2 as oxidative agent

Formation of DBPs is an issue of major concern due to the fact that these DBPs are potentially hazardous substances for health. When ClO_2 is used as disinfectant, the major risk is posed on the formation of inorganic by-products including chlorite (ClO_2^-) and chlorate (ClO_3^-) (WHO, 2004). These are formed due to the reduction of ClO_2 to chlorite when it reacts with organic matter. ClO_3^- is formed in much lower concentration than ClO_2^-; approximately 50%-70% and 30% of consumed ClO_2 is transformed to ClO_2^- and ClO_3^- respectively (EPA, 1999). These inorganic by-products can lead to hemolytic anemia at low levels of exposure and higher levels can result in an increase in methemoglobin. Additional studies have shown that chlorite can produce effects on the nervous systems in infants and young children (Veschetti et al., 2004).

There are several post- treatment techniques that can remove chlorite and ClO_2 residuals from treated water. However, these post-treatments are not valid for chlorate since once it is formed, it is stable in water and thus it cannot be removed. These post-treatment techniques are i) addition of reduced-sulfur compounds (e.g. sulfur dioxide and sodium sulfite); ii) Granular activated carbon (GAC) or powdered activated carbon (PAC); iii) adding reduced iron salts (e.g. ferrous chloride and ferrous sulfate) (EPA, 1999). However, the most convenient method is to use reduced iron salts since it has already been proved to be an effective method to remove chlorite with chloride as expected byproduct (Sorlini & Collivignarelli, 2005; Katz & Narkis, 2001). The reduction of iron salts to chloride is governed by the following equation:

$$4Fe^{2+} + ClO_2^- + 10H_2O \rightarrow 4\ Fe(OH)_{3(s)} + Cl^- + 8H^+ \quad \text{(Eq. 3)}$$

According to Sorlini & Collivignarelli (2005), complete removal of chlorite can be achieved with a stochometric dose of ferrous ion 3,31 mg Fe^{2+}/mg ClO_2^- (Katz & Narkis, 2001) at neutral pH (6.5-8.0). This reaction is kinetically fast with complete ClO_2^- reduction applying reaction times of 5 – 15 seconds. It is also possible to remove ClO_2 left in solution by the following ClO_2/ $Fe(aq)^{2+}$ reaction:

$$4H^+ + ClO_2 + 5Fe^{2+} \rightarrow Cl^- + 5Fe^{3+} + 2H_2O \quad \text{(Eq. 4)}$$

Therefore the theoretical Fe^{2+} for the complete reduction of 1mg ClO_2 is 4.14 mg of Fe^{2+}.

Formation of significant concentrations of organo-halogen and non-halogenated DBPs has not been extensively reported in previous studies. However, a study performed by Richardson et al. (2010) detected organic DPBs after the treatment of drinking water with ClO_2. From all the 27 detected organic DPBs only two of them were chlorinated DPBs, meanwhile the others were non-halogenated DPBs (ketones, carboxylic

acids and maleic acids). Another study performed by Serrano et al. (2015) states that four brominated HAAs (haloacetic acids) were formed after disinfection of drinking water with ClO_2 due mainly to the reaction of organic matter with bromide present in the water but any THMs were detected.

3.3. Previous studies with ClO_2

There are several studies that show promising results using ClO_2 to treat drinking water, surface water and wastewater.

Huber et al. (2005) studied the performance of ClO_2 during the oxidation with pharmaceuticals in water treatment. The oxidation experiments were performed in "natural waters", surface water and groundwater, where the samples were spiked with pharmaceuticals with concentrations up to 1μg L^{-1}. Results for this study show that diclofenac, one of the pharmaceuticals that have been recently included in the "watch list" of the Water Framework Directive and that has a low removal level by conventional methods, was completely removed by oxidation with ClO_2. According to this study, diclofenac was oxidized in surface water samples by more than 90% within 1 minute at the lowest tested concentration of 0.5 mg ClO_2 L^{-1}. Lee et al. (2010) studied the kinetics of different oxidants in wastewater samples from effluent of secondary treatment spiked with different pharmaceuticals. The consumption kinetics and decay pattern of ClO_2 was similar to chlorine; they showed an initial phase with a rapid consumption of oxidant within the first 2 minutes and then a slow decrease over 60 minutes of reaction time. On the other hand, ozone was totally depleted in less than 2 minutes. Andersen (2010) studied the oxidation of ClO_2 in wastewater effluent spiked with three steroid estrogens (E1, E2 and EE2) to a concentration of 0.4 μg L^{-1}. This study demonstrates that the three tested steroid estrogens (E2 and EE2 belongs to the recent "watch list" of emerging pollutants) were removed within the first 30 seconds with 2.5 mg/L and 3.75 mg/L of ClO_2 for low COD (~ 15 mg/L) and high COD (~ 40 mg/L) effluents respectively without leaving ClO_2 residuals. This may be due to the fact that steroid substances contain phenolic groups to which ClO_2 is a highly selective oxidant. Another study performed by Hey et al. (2012) examined the removal of 56 different pharmaceuticals spiked in biologically treated wastewater effluents after 18 hours of reaction. In the effluent with low COD (35 mg/L) more than a half of the studied pharmaceuticals were removed by more than 90%, meanwhile one third of the pharmaceuticals resisted degradation even at the higher tested ClO_2 concentration (20 mg/L). This group of pharmaceuticals characterized by being less sensitive to be oxidized by ClO_2 included β-blockers such as metropolol and bisoprolol which have a secondary amine functional group. The most easily oxidized pharmaceuticals (ClO_2 concentration between 0.5mg/L and 1.25 mg/L) were diclofenac, hormones containing phenolic structures (estriol, estrone, ethinyl) and antibiotics like ciprofloxacin.

It can be said that most of the previous studies (Table 1) have mainly tested the disinfection effect of ClO_2 in both drinking water and wastewater. During the last years, more research has been conducted in ClO_2 as oxidant for pharmaceuticals but this has been focused on how (kinetics) and which pharmaceuticals can be removed according to different oxidant concentration as well as the formation of inorganic by products. None of them has however studied how to optimize this technique in order to upscale it and achieve the maximum removal of pharmaceuticals.

Table 1. Previous studies in ClO_2 treatment.

Application	Size	Reference
ClO_2 kinetics in drinking water	Batch test	Hoigné and Bader, 1994
Drinking water treatment	Batch test	Korn, 2002
Inorganic DBPs in wastewater	Pilot plant	Veschetti et al. 2004
Oxidation of pharmaceuticals in natural waters	Batch test	Huber et al. 2005
Organic DBPs in drinking water	Full/pilot scale	Richardson et al. 2010
Oxidation of micropollutants in wastewater (kinetic transformations)	Batch test	Lee et al. 2010
Oxidation in spiked (steroid estrogens) wastewater	Batch test	Andersen 2010
Oxidation pharmaceuticals in spiked wastewater	Batch test	Hey et al. 2012, 2013
Organic DBPs in drinking water	Full scale	Serrano et al. 2015

3.4. Motivation and possibilities for ClO_2 treatment in WWTPs

Even though chlorine and ClO_2 approximately react with the same compounds (Huber et al. 2005), ClO_2 is much preferred due to the toxicity of DBPs generated by the use of chlorine; actually more than 300 DBPs have been addressed due to disinfection with chlorine (Richardson et al., 2000). Besides ClO_2 is more selective than chlorine in water and thus less doses are needed to have the same effect (WHO, 2004).

When comparing ClO_2 and ozone, both chemical oxidants react with electron-rich groups of organic molecules such as phenolic- and aminogroups. However it has been seen that ozone reacts faster with the same reactive functional group (Huber et al. 2005, Hey, 2013). Regarding the formation of by-products and their toxicity, it is known that inorganic by-products are formed during the treatment with ClO_2 (e.g chlorite). The formation of other significant disinfection byproducts such as organo-halogen and non-halogenated by-products is not very well known yet. In the case of ozonation, there is a risk for the formation of both organohalogen by-products (e.g bromoform with presence of bromide ion) and non-halogenated by-products (e.g aldehydes, ketoacids), as well as inorganic by-products (e.g bromated, iodate) (WHO, 2004).

Regarding the cost of these technologies, treatment with ozone has a large capital cost of 100-300 k€ for a medium size WWTP. This high cost may not be reasonable and profitable for smaller WWTPs although it could be acceptable for larger WWTPs. On the other hand, ClO_2 generators are simpler and cheaper (its cost is around 10-30 k€) for both the preparation system and the reaction chamber (Andersen, 2010). However the cost of producing ClO_2 can be the same or double compared to ozone depending on the chemicals that are used to produce the ClO_2 solution and the scale of production. Therefore the best approach could be to use ClO_2-treatment for upgrading small scale WWTPs (<2000 pe) since the capital cost for ClO_2 technology is lower than for ozone and the running costs would not be so high considering treatment for limited period of time and lower scale of consumption.

Previous studies in ClO_2 treatment (Table 1) showed that the optimization of this treatment focused on pharmaceuticals treatment for upscaling has not been deeply studied yet. Therefore, this study aims to test not only the influence of different doses of oxidant in pharmaceuticals removal but also the reaction time. This is a key factor for the design of reactor (e.g continuous stirred-tank reactor) since it allows calculating its volume once the residence time of the chemical is known.

One additional key issue that needs to be faced when thinking in upscaling a new treatment technology to pilot or full scale is that composition and inflow of wastewater varies within time. Thus, dosage control is important in order to avoid high cost and achieve good resource-efficiency within the process.

The hourly variation of flow and normalized concentration of pharmaceuticals substances coming into the Linköping WWTP was studied by Sehlén et al. (2015) (Fig. 5). The mass flow of pharmaceuticals follows the water flow in a large extent and they reach a minimum value around 7am. This shows that the dosing of the oxidant for pharmaceuticals removal can be adjusted according to the mass flow in full scale process. As an online measurement of pharmaceuticals is not possible due to their low concentration in water, this study performed by Sehlén et al. 2015 proposes ozone dosage control by measuring chemical oxygen demand (COD) or Spectral Absorption Coefficient (SAC). COD was not considered a suitable indicator in this case because it is not robust for real-time measurements. Decrease of SAC, which is the absorbance at 254nm at which organic matter can be quantified, was tested as a parameter that can be linked to the removal effect of the oxidant substance. There is not any relationship between pharmaceuticals concentration and ultraviolet absorbance since they constitute a very small proportion of the total amount of substances absorbing at 254nm. This SCA could be monitored on line and thus the dosage of the oxidant for the removal of pharmaceuticals could be adjusted leading to a more resource efficient process implementation.

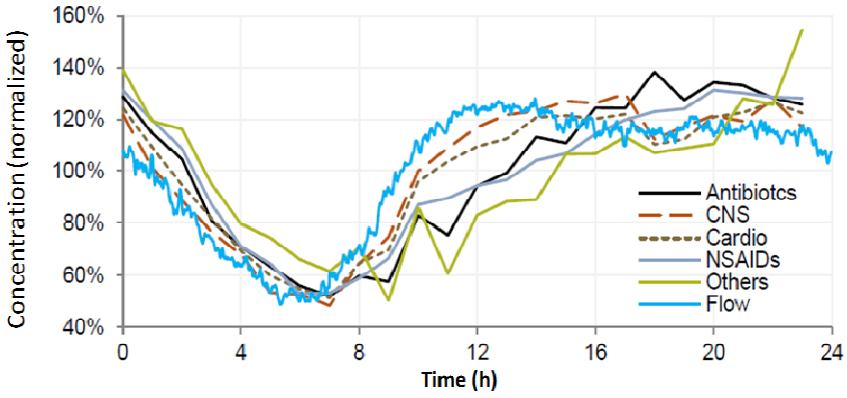

Fig. 5. Hourly variation of mass flow of pharmaceuticals in Linköping WWTP (Shelén et al. 2015).

4. MATERIALS AND METHODS

This section aims to present the chemicals, analytical methods and experimental setup defined for the current study.

4.1. Chemicals

ClO_2 stock solution of 500mL (AquaCare®) was provided by Xinix AB and stored in an amber bottle glass at 4ºC. This product is currently used as a safe protection against pathogenic microorganisms (bacteria, viruses) in water. It can be also used for personal hygiene and disinfection of food. This solution was obtained from a reaction between 8,2%$NaClO_2$ and 9,8%HCl to achieve a ClO_2 concentration of approximately 1500ppm.

A stock solution of Na_sSO_3 was synthesized by adding 10 g of Na_sSO_3 (Sigma–Aldrich, Steinheim, Germany) to 100 mL of deionized water to get a concentration of 100g/L. This stock solution is used to quench the oxidation reaction.

4.2. Analytical methods

4.2.1. Chlorine dioxide analysis

The concentration of residual ClO_2 is measured by standardized 100608 Spectroquant® ClO_2 test that quantifies ClO_2 concentration by reaction with DPD (N-diethyl-p-phenylenediamine) and later on using a WTW photolab 6600 UV-VIS spectrophotometer. This method allows measuring in a range of concentrations from 0.02 to 10 mg/L ClO_2 in rectangular cells (10, 20 and 50mm).

4.2.2. Ultraviolet absorbance measurement

Ultraviolet absorbance at 254nm was measured in 10mm rectangular cells using WTW photolab 6600 UV-VIS spectrophotometer. This was done in order to be able to detect organic matter in water. Water samples were filtered through glass microfiber disc (Munktell®) before measuring in order to avoid colloidal solids scattering UV light.

4.2.3. Microtox test

Microtox tests were done using 90% Basic Text method which is designed for use with samples of low to medium toxicity (e.g. wastewater effluent) where the response of toxicity is measured as a change in luminescence of bacteria. Microtox Acute Reagent with bacteria was added to 9 different vials containing water and NaCl solution to achieve a final salinity of 2% and thus diluted in 9 different concentrations. Initial measurements with only bacteria were carried out in order to have toxicity blank values. Then, the sample was added to the vials resulting in 9 different sample concentrations. Finally, the measured light was correlated with the toxicity of the samples and data was generated for the calculation of EC_{50} values both after 5min and 15min.

4.2.4. Pharmaceuticals analysis

The concentration of pharmaceuticals in the wastewater samples was analyzed at IVL Swedish Environmental Institute according to a defined calibrated method that includes 23 different pharmaceuticals (Table 2). These pharmaceuticals have frequently been found wastewater effluents in Sweden and they cover a broad range of pharmaceuticals concerning different mode of actions (NSAIDs, antidepressants, antihypertensives, sedatives), different functional groups (phenol, tertiary and secondary amine, aniline, fluoro, keto, organosulfur) and different $LogK_{OW}$ values.

Table 2. The 23 pharmaceuticals analyzed in wastewater in the present study.

Nr	Substance	Abrev	Mode of action	Nr	Substance	Abrev	Mode of action
1	Amlodipine	*Amlo*	*Antihypertensives*	13	Naproxen	*Naprox*	*Anti-inflammatories*
2	Atenolol	*Ateno*	*Antihypertensives*	14	Oxazepam	*Oxa*	*Sedatives*
3	Bisoprolol	*Bisop*	*Antihypertensives*	15	Paracetamol	*Parac*	*Anti-inflammatories*
4	Carbamazepine	*Carba*	*Sedatives*	16	Propranolol	*Prop*	*Antihypertensives*
5	Citalopram	*Citalo*	*Antidepressants*	17	Ramipril	*Rami*	*Antihypertensives*
6	Diclofenac	*Diclof*	*Anti-inflammatories*	18	Ranitidine	*Rani*	*Antiulcers*
7	Fluoxetine	*Fluox*	*Antidepressants*	19	Risperidone	*Risp*	*Antipsychotic*
8	Furosemide	*Furos*	*Diuretics*	20	Sertralin	*Sert*	*Antidepressants*
9	Hydrochlorothiazide	*Hydrochl*	*Antihypertensives*	21	Simvastatin	*Simv*	*Lipid-regulating*
10	Ibuprofen	*Ibu*	*Anti-inflammatories*	22	Terbutaline	*Terb*	*Asthma medication*
11	Ketoprofen	*Keto*	*Anti-inflammatories*	23	Warfarin	*Warf*	*Anticoagulants*
12	Metoprolol	*Meto*	*Antihypertensives*				

More details about these substances (chemical structure, ion mode, retention time, recovery, LOD and LOQ) can be seen in Appendix I.

Solid phase extraction

The extraction of pharmaceuticals from wastewater was carried out according to a previous method defined by Gros et al (2006) for multi-residue analysis of pharmaceuticals in wastewater. Before solid phase extraction (SPE), samples of 100mL were spiked with 50 μL of the internal standards (IS) Carbamazepine-13C15N (2μg/mL) and Ibuprofen – D3 (2μg/mL). These are isotopic labeled internal standards what mean that they are chemically identical with the target analyte, bur with a slight difference in mass. Besides both blank and standard samples were prepared with tap water and they were also spiked with the IS. The standard sample was spiked with 100 μL of a mix solution (100μg/L) that contained the 23 pharmaceuticals (Table 2). Then, 200mg of EDTA-Na_2 was added to the samples and shaked at 120 rpm during 30 minutes. Pharmaceutical´s analytes were extracted from the water samples using SPE cartridge (Oasis HLB, 6cc, Waters). This column was first conditioned with 10 mL of methanol followed by 10 mL of MQ-water. After conditioning, wastewater sample was added to the column at a flow of 2 drops/second (approximately 10 mL/min). The analytes were eluted and collected from the column adding 5mL of a solution methanol:water (1:1) followed by 5mL of acetone. Thereafter, the elute was evaporated to dryness with a gentle stream of nitrogen gas at 40ºC. The analytes were then reconstituted in 1 mL of 0,1wt% EDTA-Na_2 solution in methanol: water (1:1). Finally, the samples were centrifuged, only in case supernatants contained suspended solid particles, in Eppendorf-tubes at 10 000 rpm for 10 minutes before the samples were transferred to the vials.

High performance liquid chromatography- triple quadrupole mass spectrometry (HPLC-MS/MS)

Determination of the amount of pharmaceuticals in the wastewater samples was carried out on a high performance liquid chromatography-triple quadrupole mass spectrometry (HPLC-MS/MS). The first step was to insert the vials into a binary liquid chromatography (UFLC) system equipped with a C18 reversed phase-column with a dimension of 50 x 3 mm and a particle size of 2.5 μm (Xbridge, Waters Corporation Milford, USA) to perform the chromatographic separation. The process was run isothermically at a temperature of 35ºC and at flow rate of 0,3 mL/min. The mobile phase consisted of 10mM acetic acid in water (mobile phase A) and methanol (mobile phase B). At the beginning of the sample injection, the gradient started with 100% of mobile phase A and 0% of mobile phase B. Then the percentage of mobile phase B increased linearly to 95% for 11 minutes and kept 95% for 5 minutes. After that, mobile phase B was decreased till 0% during 1 minute and kept for 3 minutes before another sample was injected. The total time for one analysis was 20 minutes. This binary liquid chromatographer was then linked to atmospheric pressure ionization (API) 400 triple quadrupole (MS/MS) (Applied Biosystems, foster City, USA) with an electrospay ionization interface that worked in both positive and negative mode. By using both positive and negative mode for ionization the sensitivity increases for the analytes since they can be either positively or negatively charged. Two fragments were measured for each pharmaceutical and eight points for calibration corresponding to a concentration range between 0 and 500 ng/L were included.

Data analysis

Once the samples were analyzed by the HPLC/MS/MS, analyte peaks were quantified according to the retention time for each pharmaceutical (Appendix I). Analyst Software version 1.6, which is a platform that enables data processing for Mass Spectrometry Systems, was used in order to obtain the area under the peak for each analyte and its two fragments. Then, the concentration of pharmaceuticals in each sample was calculated by external calibration using the concentrations provided by the eight points of calibration. The calculated concentration was based on the recovery of the analyzed substances; this means that the concentration for each pharmaceutical was divided by a percentage of recovery that was calculated according to the amount of standard solution that had been recovered after the solid phase extraction.

In order to take into account limits in the analytical method concerning accuracy, precision and sensitivity to the analyzed substances, two different analytical limits have been calculated for each pharmaceutical (Appendix I). Limit of detection (LOD) is the lowest analyte concentration in a sample that can be reliably found. LOD has been calculated as:

$$LOD = 3x\ mean_{blank} \quad \text{(Eq. 5)}$$

The second analytical limit is LOQ or limit of quantification. It refers to the lowest concentration of analyte that can be quantified in a sample, while meeting some predefined goals for bias and imprecision. This analytical limit has been calculated as:

$$LOQ = 3{,}33xLOD \quad \text{(Eq.6)}$$

4.3. Experimental setup

4.3.1. Wastewater effluent

Effluent wastewater from Henriksdal WWTP was collected at the Hammarby Sjöstadsverk R&D facility to perform the experiments. Henriksdal STP is one of the largest WWTPs in Sweden and it treats wastewater from approximately 720 000 people. Wastewater in this facility is treated mechanically by screening and grit chambers, followed by chemical pre-precipitation with ferrous sulphate. Then, wastewater is biologically treated by an active sludge predenitrification process followed by a secondary precipitation with ferrous sulphate to act as flocculant before going through the sand filters which is the last treatment of the process.

20 L of wastewater were collected at one time in 1 L plastic bottles in order to have the same characteristics within the wastewater (pH, COD, N, etc.) and same concentration of pharmaceuticals during the performance of the batch tests.

Wastewater with a higher COD compared to the treated wastewater from Henriksdal was collected at the influent of the pilot-scale deammonification process comprising two moving bed biofilm reactors at Hammarby Sjöstadsverk. This wastewater was filtered through glass microfiber disc (Munktell®) before measuring its characteristics.

To characterize the wastewater, pH, COD and total nitrogen (Table 3) were measured spectrophotometrically by standardized test kits WTW 14540 and WTW 14537 respectively.

Table 3. Characteristics of analyzed wastewater.

	pH	COD (mg/L)	N-tot (mg/L)
Effluent Henriksdal	7.0	21	8.8
Influent deammonification	7.7	60	46

4.3.2. Methodology to find optimal ClO_2 dose- reaction time

As mentioned before, one of the main goals of the study was to find the optimal ClO_2 dose/reaction time in order to achieve as high removal of pharmaceuticals as possible. The methodology that has been followed in this study can be summarized in 6 different steps (Fig. 6).

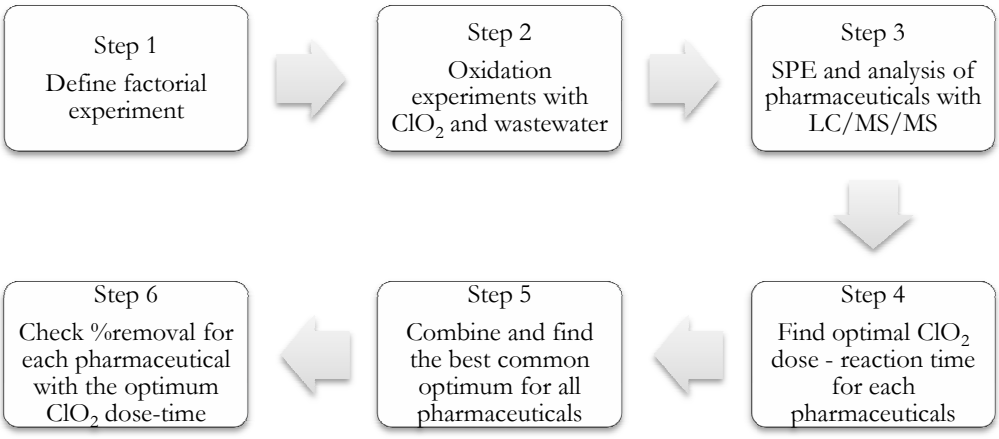

Fig. 6. Methodology to find optimal ClO2 dose – reaction.

Design of factorial experiment

First of all, a "factorial design" approach was selected as the best option to achieve the optimal dose-reaction time. The difference compared to the traditional experimental design or "one factor at-a-time" (OFAT) is that factorial designed experiments allow changing different parameters at the same time within a fix number of experimental tests which means a resource-efficient way to design experiments (Korn, 2002). The factorial experiment proposed in this study comprises two factors (ClO_2 dose and reaction time) as well as three different levels (low, medium, high). This means that individual 3^2 (3 levels, 2 factors) factorial experiments were carried out (Table 4). The response of this factorial experiment will be the percentage of removal for each pharmaceutical.

Table 4. Factorial experiment description a) and tested samples b) (Step 1 in Fig.6).

a)

Factors	ClO_2 dose (mg/L)			Reaction time (min)		
Level	-1	0	1	-1	0	1
Value	**0.5**	**10**	**20**	**5**	**30**	**60**

b)

Sample	Concentration (mg/L)	Time (min)
1	0,5	5
2	0,5	30
3	0,5	60
4	10	5
5	10	30
6	10	60
7	20	5
8	20	30
9	20	60

In order to quantify the 3 levels for the 2 different factors, previous studies were consulted. In the study performed by Hey et al. (2012) it is possible to see the profile of ClO_2 consumption with time in a biologically treated wastewater (Fig 7). It shows that with a reaction time higher than one hour the remaining ClO_2 concentration is constant meanwhile during the first six minutes the concentration of the oxidant has already decreased one-third of the initial concentration. Accordingly to this data, the low, medium and high levels for the reaction time factor were set as 5, 30 and 60minutes respectively. The quantification of the levels of ClO_2 doses were also set accordingly to the previous study performed by Hey et al. (2012) where a range of 0.5-20 mg/L ClO_2 was tested. This study states that it is possible to remove some pharmaceuticals with the lowest dose of 0.5 mg/L and those that cannot be removed with the highest ClO_2 concentration of 20 mg/L can be considered non-oxidizable. Therefore the low, medium and high levels for the ClO_2 dose factor were set as 0.5, 10 and 20mg/L respectively.

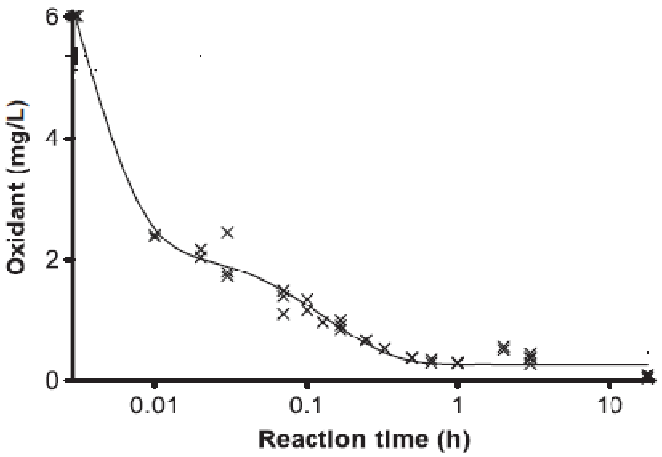

Fig. 7. Profile of ClO2 consumption with time in a biologically treated wastewater (Hey et al. 2012).

Oxidation experiments

The oxidation experiments were made with the stock ClO_2 solution and the effluent wastewater from Henriksdal WWTP. Experiments were conducted at bench-scale using 100 mL glass bottles serving as batch reactors (Fig.8).

The bottles were previously covered by aluminium foil in order to avoid light interfering in the oxidation reaction since ClO_2 is quite sensitive to sunlight. Aluminium foil was also set between the cap and the glass bottle in order to prevent plastic cap to contaminate the sample and adsorb chemicals from the sample. Then, 100 mL of wastewater from Henriksdal effluent were set in each of the 9 bottles and labeled accordingly to Table 4. This means that every bottle represents a different pair of concentration-time values in order to perform the proposed factorial experiment. The volume of ClO_2 solution added to each sample was calculated according to the concentration of the stock ClO_2 solution. This concentration was previously checked before the performance of the experiments by ClO_2 analysis with the spectrophotometer and its measurement kit as stated before (section 4.2.1) . Once the ClO_2 solution was added to the wastewater samples, bottles were placed into a shaking table at ~ 120 rpm in order to have a proper mix of ClO_2 solution with the wastewater. When the reaction time was reached for each sample, pH and ClO_2 concentration was measured. The residual oxidants were removed by the addition of 200 mg/L Na_sSO_3 in order to stop the oxidation reaction at the proper time defined for each sample. The samples were then stored in the fridge at 4ºC until analysis of pharmaceuticals was carried out.

Fig. 8. Batch experiment with labeled 100mL glass bottles. From left to right (5C1, 30C1, 60C1, 5C2, 30C2, 60C2, 5C3, 30C3, 60C3).

Once oxidation experiments were performed, pharmaceuticals were analyzed for each one of the 9 samples plus one no treated sample as stated in section 4.2.4. This allows calculating the removal percentage for each pharmaceutical by the following equation:

$$\eta = \frac{C_o - C_x}{C_o} \cdot 100 \quad \text{(Eq. 7)}$$

Where η is the degradation efficiency, C_o is the initial concentration of the pharmaceutical and C_x is the concentration of pharmaceutical left after treatment with ClO_2.

Optimal ClO_2 dose-time with MODDE

In order to find the optimal ClO_2 dose-reaction time for each pharmaceutical, MODDE application version 10.1 is utilized. ClO_2 dose and reaction time are set as independent factors, meanwhile the response is the percentage of pharmaceuticals removal or degradation efficiency (η). The response surface modeling (RMS) was selected as model type together with a face centered central composite design (CCF) which is the preferred choice for optimization when systems involving two to five factors are mapped with three levels for each factor (Eriksson et al., 2008). The output of this optimization process was ClO_2 dose-reaction time values that gave the highest possible percentage of pharmaceuticals removal using the least possible resources (ClO_2 dose and time). This optimization process was repeated for each one of the pharmaceuticals present in the wastewater and the target removal was adjusted individually for each pharmaceutical

Then, all these optimum values (ClO_2 dose-time-%removal) were integrated in MODDE in order to find one common optimum dose-time for all pharmaceuticals. The last step was to check the percentage of removal for each pharmaceutical that is reached with the obtained optimum dose-time.

This was an iterative process in the sense that the range of the one of the factors can be narrowed in order to find a more accurate optimum.

4.3.3. Relation between pharmaceuticals removal and ultraviolet absorbance (UVA254nm)

The goal in this part was to investigate if there is some correlation between pharmaceuticals removal and ultraviolet absorbance at 254 nm left after the treatment with different doses of ClO_2. In order to do that, seven different concentrations of ClO_2 in a range from 20 to 0mg/L with constant reaction time were tested in the wastewater. Once the reaction was completed both UVA254nm (section 4.2.2) and concentration of pharmaceuticals (section 4.2.4) were measured.

4.4. Ranking of pharmaceuticals according to their environmental impact indicator

Analyzed pharmaceuticals were ranked according to an environmental indicator that allows classifying them accordingly to the risk that they pose for the ecosystem health (e.g. effects on aquatic organisms living downstream WWTPs). This was done in order to prioritize those substances with higher environmental risk and thus create a priority list of pharmaceuticals.

The environmental indicator used to rank the pharmaceuticals is based on a comparison between environmental concentration (EC) and predicted non effect concentration (PNEC). If the ratio between them or predicted ecotoxicological effect risk (EC/PNEC) has a value of 1 or higher, the substance would pose a high risk for the environment. Those

values in between 0.1-1 are classified as medium risk, meanwhile substances with an environmental risk ratio below 0.1 would pose a low risk for the ecosystem health.

EC is the measured concentration of pharmaceuticals in analyzed untreated samples from effluent wastewater at Henriksdal WWTP. These samples are supposed to provide a good description of the output levels of pharmaceuticals at the recipient since there is no significant reduction after the biological treatment (Wahlberg et al., 2010).

The predicted no effect concentration (PNEC) can be calculated as:

$$\text{PNEC} = \frac{\text{NOEC} \cdot \text{Dilution factor}}{\text{Assessment factor}} \qquad (\text{Eq. } 8)$$

where NOEC is the no effect concentration or the lowest concentration of a substance that does not have any toxic effect on the aquatic environment.

Assessment factor takes into account the sensitivity between individuals of the same species, different trophic levels and habitats. The more sensitive a test is, which means that a larger number of tests in several species and different trophic levels have been performed, the lower is the assessment factor. This factor can have values between 10 000 and 10 depending on how sensitive the test is.

Both NOEC and assessment factors were retrieved from the Pharmaceutical Specialties in Sweden (FASS) drug portal where a database with information about ecotoxicological studies for different pharmaceuticals can be found.

Dilution factor is applied to calculate the PNEC in the recipient and to be comparable with the EC. In this study a dilution factor of 10 is applied for the calculation of PNEC according to ECHA (2008).

5. RESULTS

First, a priority list for those pharmaceuticals detected in the effluent wastewater from Henriksdal WWTP is presented. Thereafter, results concerning the removal of pharmaceuticals after the oxidation experiments are addressed together with the evaluation of optimal ClO_2 dose- reaction time. Then, residual concentration of ClO_2 after oxidation tests is studied as well as the relationship between UVA254nm and removal of pharmaceuticals.

5.1. Priority list of pharmaceuticals

20 different pharmaceuticals from those with a previous defined calibrated method (Table 2) were detected in the effluent wastewater from Henriksdal WWTP. The concentration of these 20 pharmaceuticals is above their corresponding LOQ which has values in between 2.58ng/L and 127.36ng/L (Appendix I) depending of the pharmaceutical. After analysis, these 20 substances have been ordered according to their impact on ecosystem health in order to create a priority list with pharmaceuticals (Table 5) that an additional ClO_2 treatment step would help to remove.

According to this classification, 60% of the pharmaceuticals found in wastewater effluent have low risk for the ecosystem meanwhile 25% and 15% of the pharmaceuticals have moderate and high risk respectively (Table 5). Those pharmaceuticals with the highest environmental risk (EC/PNEC) among the studied substances are oxazepam, diclofenac and metoprolol.

Table 5. Priority list of pharmaceuticals classified according to their environmental risk ratio (EC/PNEC). High, medium and low risk are represented in red, yellow and green color respectively.

	Pharmaceutical	EC (µg/L)	NOEC (µg/L)	Assessment factor	Dilution factor	PNEC (µg/L))	EC/PNEC
High risk	Oxazepam	0,356	1,80	1000	10	0,018	19,78
	Metoprolol	1,025	1,00	50	10	0,2	5,12
	Diclofenac	0,605	0,50	10	10	0,5	1,21
Medium risk	Fluoxetine	0,018	0,03	10	10	0,029	0,64
	Propanolol	0,061	0,50	50	10	0,1	0,61
	Carbamazepine	0,276	1,00	10	10	1	0,28
	Amlodipine	0,022	10,00	1000	10	0,1	0,22
	Furosemide	1,508	142,00	100	10	14,2	0,11
Low risk	Naproxen	0,555	32,00	50	10	6,4	0,09
	Ketoprofen	0,293	1041,00	1000	10	10,41	0,03
	Citalopram	0,198	105,00	100	10	10,5	0,02
	Sertralin	0,043	9,00	50	10	1,8	0,02
	Paracetamol	0,026	30,00	100	10	3	0,01
	Ibuprofen	0,036	10,00	10	10	10	0,0036
	Terbutaline	0,008	240,00	1000	10	2,4	0,0032
	Atenolol	0,220	1000,00	100	10	100	0,0022
	Ranitidine	0,147	310,00	50	10	62	0,0024
	Warfarin	0,012	59,00	100	10	5,9	0,0021
	Bisoprolol	0,064	1780,00	50	10	356	0,0002
	Hydrochlorothiazide	1,034	10000,00	10	10	10000	0,0001

There are some pharmaceuticals (Fig. 9) with a high EC (e.g. hydrochlorothiazide) but that pose a low environmental risk; meanwhile there are others such as fluoxetine that has a low EC but its environmental risk is moderate. This is due to the fact the environmental risk ratio (EC/PNEC) depends on NOEC and assessment factor values, thus those substances with a low PNEC (e.g. fluoxetine, oxazepam) usually pose greater risk for the ecosystem health than those with higher PNEC values (e.g. hydrochlorothiazide). Some of the lowest concentrations in the effluent wastewater (Fig. 9) belong to ibuprofen and paracetamol, which are two pharmaceuticals whose concentrations in the incoming water to a WWTP are usually very high. However, it is known that both ibuprofen and paracetamol can be almost totally degraded during conventional treatments in a WWTP (Fig. 3) and therefore they present low concentrations at the effluent.

5.2. Optimal ClO_2 dose- reaction time

This section aims to describe the results obtained from the factorial experiments in order to find the optimal ClO_2 dose – reaction time to achieve the maximum removal of the pharmaceuticals analyzed in the effluent wastewater. This optimum has been calculated for 17 of 20 pharmaceuticals detected in the water. The three pharmaceuticals that have not been included in this optimal study are paracetamol, terbutaline and warfarin. This is because their concentrations in untreated samples are below two times their LOQ (34.89 ng/L, 8.80 ng/L and 7.45 ng/L respectively) so they are not considered representative to calculate their respective optimal ClO_2 dose –time.

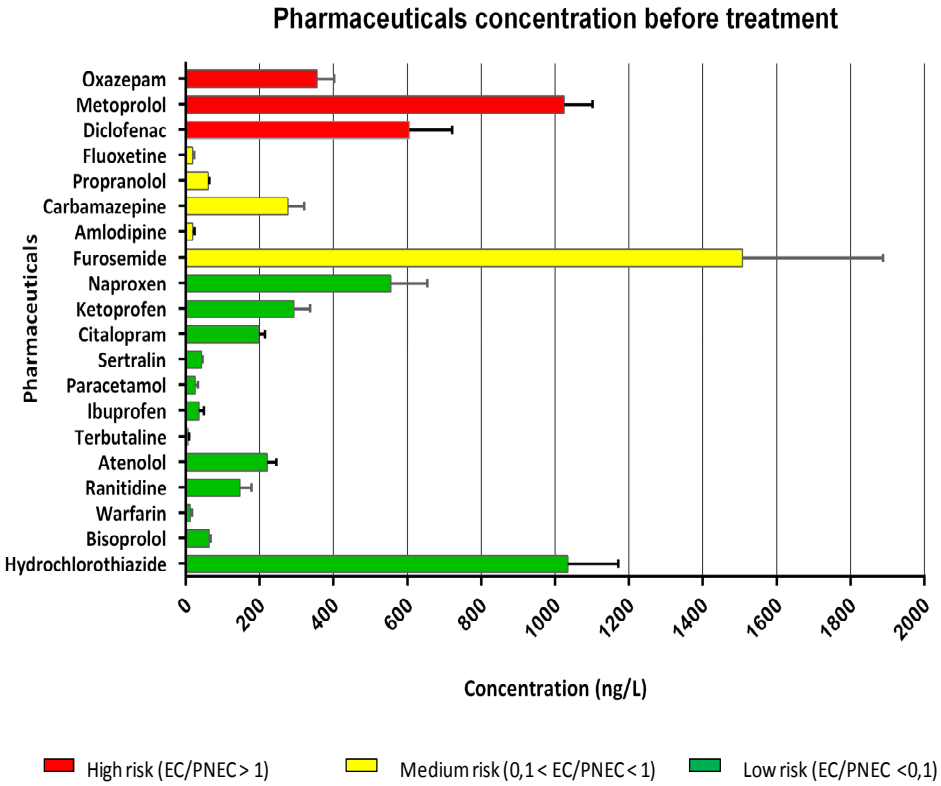

Fig. 9. Pharmaceuticals analyzed at the effluent of Henriksdal WWTP and classification according to their impact in ecosystem health; high, medium and low risk are represented in red, yellow and green colors respectively. Average and standard deviation calculated with 5 samples.

5.2.1. First factorial experiment

This section presents the results from the first factorial experiment where three different ClO_2 doses and reaction times (Table 4) were tested.

Optimal dose-time for each pharmaceutical

Once the oxidation experiments were carried out and removal percentages calculated for each sample and pharmaceutical (Appendix II-Table 1), optimal ClO_2 dose-reaction times were calculated with MODDE for each of the analyzed substances. The 3D scatter plot (Fig. 10) shows that optimal dose – time - removal is strongly dependent on the type of pharmaceutical; those substances located at the top of the diagram can almost be totally removed (>90%) with their respective optimal ClO_2 dose –time, meanwhile those located at the bottom of the diagram have poor removal with ClO_2 (e.g. carbamazepine, ketoprofen, oxazepam). Focusing on those pharmaceuticals that pose a relevant environmental risk for the ecosystem health, only oxazepam with high environmental risk and carbamazepine with moderate risk are not possible to be completely oxidized with 30% and 10% of removal respectively (Appendix III).

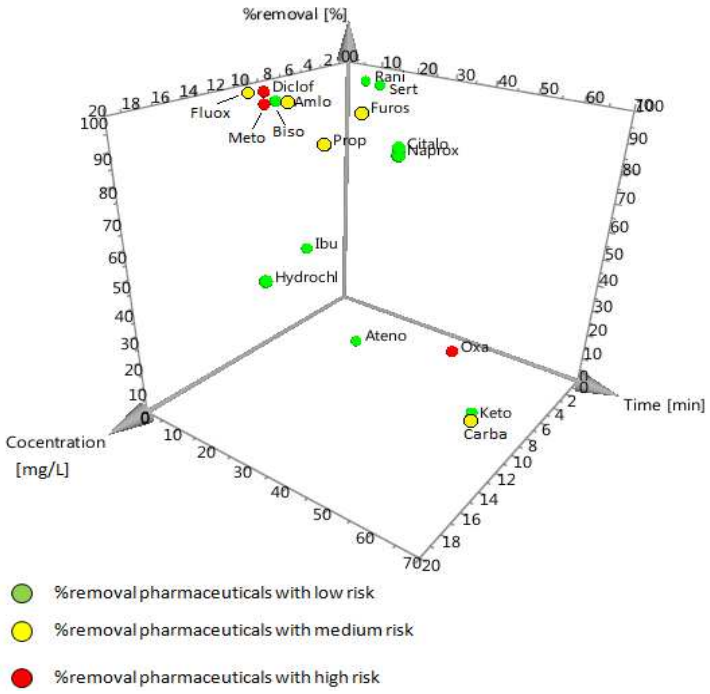

Fig. 10. Optimal ClO2 dose-reaction time for each pharmaceutical retrieved from first factorial experiment (Step 4 in Fig.6). For instance, optimal removal of ibuprofen 49% was obtained using 10 mg ClO2/L and 10 minutes.

Common optimal dose-time for all pharmaceuticals

Once all optimal ClO_2 dose – time were combined in MODDE (Fig. 11), then a common optimum for all studied pharmaceuticals was obtained (Table 6). Eight different optimum values were retrieved from MODDE although the one with the most equalized factor contribution from both dose and time was selected. According to the contour plot (Fig. 11), two areas of maximum removal were obtained. However, the selected optimal ClO_2 dose-time was located at the left bottom area of the contour plot since this zone includes the lowest doses and reaction times values that can maximize the removal of pharmaceuticals.

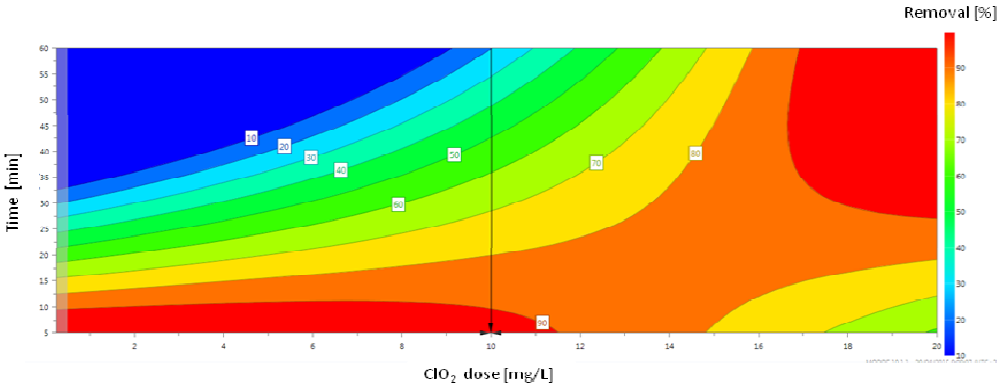

Fig. 11. Contour plot for common optimal analysis for all studied pharmaceuticals from first factorial experiment retrieved from MODDE (Step 5 in Fig.6). Black arrows indicate where the optimal dose – time – removal is located. Maximum removal belongs to red areas meanwhile lower removal is represented by blue areas.

Table 6. Common optimal ClO_2 dose-reaction time from first factorial experiment. Both values and factor contribution were retrieved from MODDE.

Common Optimum 1			
ClO_2 dose (mg/L)		Reaction time (min)	
Value	Factor contribution	Value	Factor contribution
5	51	10	49

5.2.2. Second factorial experiment

The optimal dose-reaction time obtained in the first factorial experiment is satisfactory in the sense that the lowest tested reaction time (5 minutes) seems to be the optimum reaction time for the removal of pharmaceuticals. However, an additional factorial experiment was performed in order to narrow the range of tested ClO_2 dose to see if it is possible to decrease the optimum dose of ClO_2. As the optimum dose in the first factorial experiment was 10 mg ClO_2/L and the lowest dose of 0.5 mg/L did not give significant removal results, this second factorial experiment was designed with three ClO_2 doses of 15 mg/L, 10 mg/L and 4 mg/L meanwhile the reaction time kept its original values of 60, 30 and 5 minutes (Table 7).

Table 7. Second factorial experiment description a) and samples tested b).

a)

Factors	ClO_2 dose (mg/L)			Reaction time (min)		
Level	-1	0	1	-1	0	1
Value	**4**	**10**	**15**	**5**	**30**	**60**

b)

Sample	Concentration (mg/L)	Time (min)
1	4	5
2	4	30
3	4	60
4	10	5
5	10	30
6	10	60
7	15	5
8	15	30
9	15	60

Optimal dose-time for each pharmaceutical

Removal percentages are individually calculated for each sample and pharmaceutical in this second factorial experiment (Appendix II- Table 2). Then, optimal ClO_2 dose –time for each pharmaceutical obtained with MODDE are represented in the 3D scatter plot (Fig. 12). It can be seen that those pharmaceuticals that had the highest removal in the first factorial experiment can now be removed (> 90%) with the lowest tested ClO_2 dose of 4 mg/L. Before, some of these pharmaceuticals had their optimum ClO_2 dose around 10 mg/L since it was not possible to remove them with lowest dose of 0.5 mg ClO_2/L. As expected, those pharmaceuticals that had poor removal in the first factorial experiment have now similar optimum dose – time – response values.

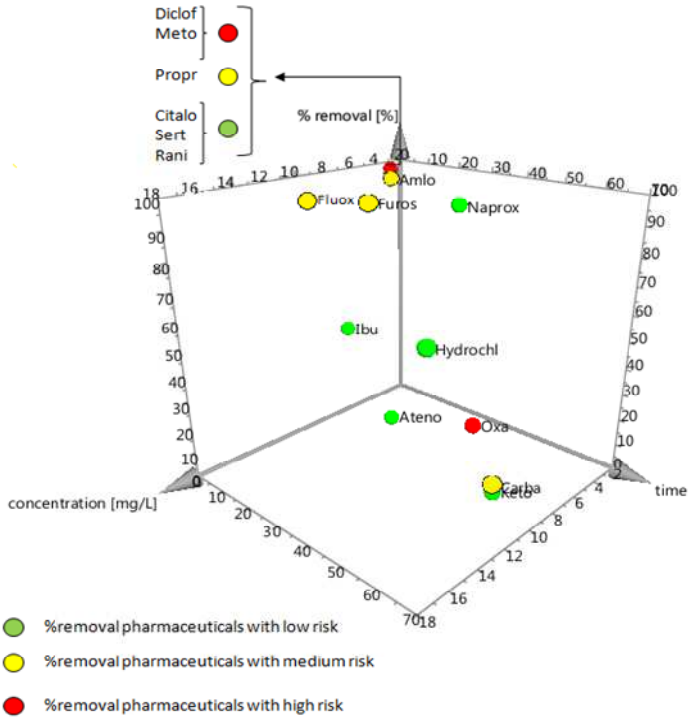

Fig. 12. Optimal ClO_2 dose-reaction time for each pharmaceutical retrieved from second factorial experiment. The arrow indicates those substances that have the same optimal dose, time and response (4mgClO2/L, 5 min, 99%).

Common optimal dose-time for all pharmaceuticals

Optimal ClO_2 dose-time for each pharmaceutical are combined with MODDE (Fig. 13.) and a new common optimum for all pharmaceuticals is obtained (Table 8). In this case, two maximum removal areas are also presented in the contour plot although the interest is focused on the area located at the left of the plot since it comprises the lowest ClO_2 doses. Again eight different optimal dose – time were retrieved from MODDE although the optimum with the most balanced factor contribution for both dose and time was selected. According to the selected optimum (Table 8), the reaction time is 5 minutes higher than the previous result (Table 6), although the optimal ClO_2 dose has decreased considerably from 10 mg/L to 5 mg/L.

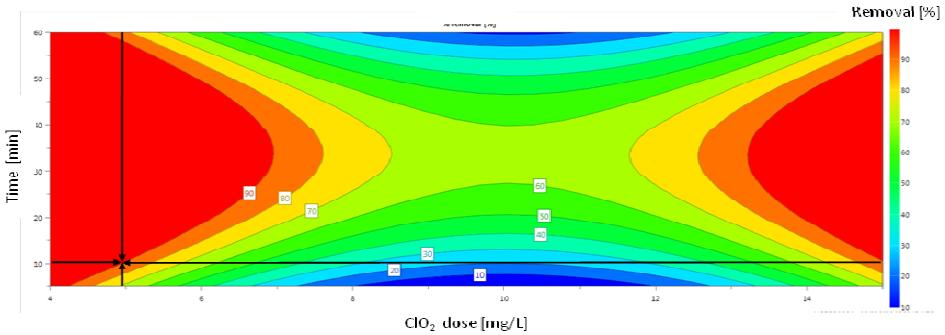

Fig. 13. Contour plot for common optimal analysis from first factorial experiment retrieved from MODDE (Step 5 in Fig.6). Black arrows indicate where the optimal dose – time – response is located. Maximum removal belongs to red areas meanwhile lower removal is red areas meanwhile lower removal is represented by blue areas.

Table 8. Common optimal ClO_2 dose-reaction time retrieved from second factorial experiment. Both values and factor contribution retrieved from MODDE.

Common Optimum 2			
ClO_2 dose (mg/L)		Time(min)	
Value	Factor contribution	Value	Factor contribution
5	54	10	46

5.2.3. Optimum for most relevant pharmaceuticals

Previous sections have evaluated the optimal ClO_2 dose-time for all pharmaceuticals detected in the effluent wastewater. However, in this section only those pharmaceuticals classified with high and moderate risk are evaluated since they are more relevant regarding their impact in ecosystem health. Thus only those pharmaceuticals marked in red and yellow color (Fig. 12) (oxazepam, metoprolol, diclofenac, fluoxetine, propanolol, carbamazepine, amlodipine and furosemide) have been taken into account for the calculations with MODDE. The result regarding optimal ClO_2 and reaction time (Table 9) for priority pharmaceuticals is quite similar to the one obtained before for the second factorial experiment (Table 8) where all the analyzed pharmaceuticals were taken into account.

Table 9. Common optimal ClO2 dose-reaction time for most relevant priority pharmaceuticals.

Common Optimum priority pharmaceuticals			
ClO_2 dose (mg/L)		Time (min)	
Value	Factor contribution	Value	Factor contribution
5	67	13	33

5.2.4. Verification of pharmaceutical removal after treatment with common optimal ClO_2 dose-time

This is the last step included in the methodology to find optimal ClO_2 dose-time (Fig. 6) and it has been based on checking the removal of each pharmaceutical individually once the common optimum was calculated. Duplicated samples were analyzed for each of the two optimums (Table 6 and 8) and removal calculated for each pharmaceutical (Appendix II-Table 3).

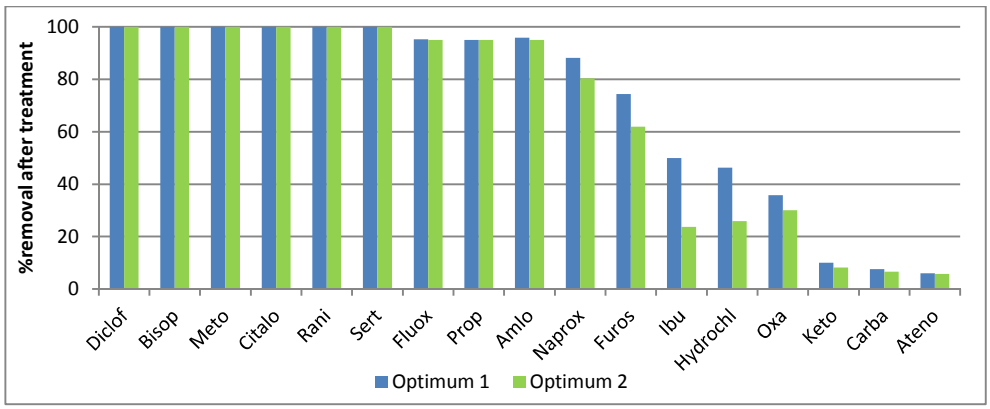

Fig. 14. %removal of analyzed pharmaceuticals with both Optimum 1 (10mgClO_2/L – 5 minutes) and Optimum 2 (5mg ClO_2/L – 10 minutes).

9 of 17 studied pharmaceuticals can be removed more than 90% when applying both optimal ClO_2 dose-time values (Fig. 14). This result implies that choosing Optimum 2 (5 mg ClO_2/L – 10min) is the best option since it is able to reach similar removal values than Optimum 1 (10 mg ClO_2 /L) but using less ClO_2 dose, thus leading to a more resource effective option.

The highest differences regarding removal efficiencies between the two optimums can be seen for ibuprofen and hydrochlorothiazide but this is not so relevant since both substances pose a low risk for the ecosystem health. The other substances have almost the same removal with both optimal ClO_2 dose – time values.

Environmental risk ratios (EC/PNEC) were calculated for each pharmaceutical after treatment with optimal ClO_2 dose – reaction time (5mg/L – 10min). Besides, final concentrations after treatment are compared with initial concentrations before treatment (Fig. 15). According to these results, oxazepam would still pose a high environmental risk after treatment with ClO_2 since this pharmaceutical cannot be totally removed with this treatment; approximately 30% of this substance could be removed from wastewater. Cabamazepine, which has one of the lowest removals (<10%), still has moderate environmental risk after the ClO_2 treatment. Other pharmaceuticals that had high risk before treatment (diclofenac and metoprolol) and moderate risk (fluoxetine, amlodipine, furosemide and propanolol) (Fig. 15) would pose now a low environmental risk after treatment with 5mg ClO_2/L-10min. Those pharmaceuticals that had a low environmental risk before treatment, still pose a low risk after treatment.

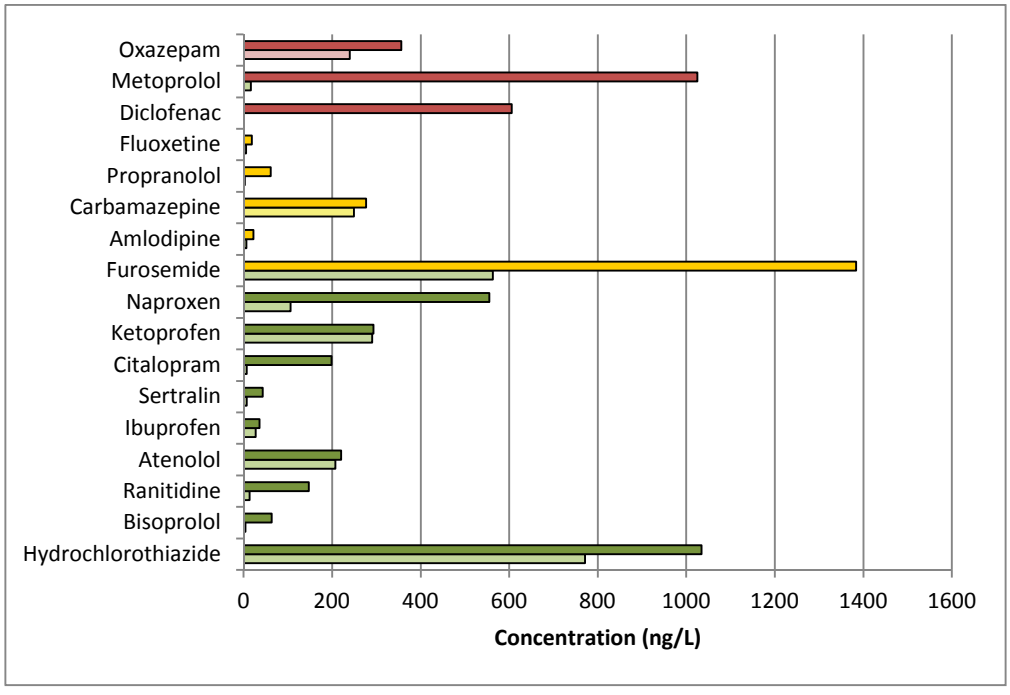

Fig. 15. Comparison of concentrations of pharmaceuticals before treatment (bar at the top of each substance) and after treatment (bar at the bottom of each substance). Dark red, yellow and green bars refer to high, medium and low environmental risk respectively before treatment, meanwhile light red, yellow and green bars refer to high, medium and low environmental risk respectively after treatment. For instance, furosemide had a medium risk (dark yellow) before treatment and low risk (light green) after treatment.

5.3. Residual ClO_2 after oxidation treatment

This section aims to present the residual ClO_2 that is left after the oxidation treatment in the wastewater samples and its dependency with time and organic matter content. No further pH adjustments were done in order to measure ClO_2 left in the solution since pH did not change significantly (6.2-6.6) after the ClO_2 addition even with the highest concentration of 20 mg/L.

5.3.1. Influence of reaction time

Residual ClO_2 concentration was measured after reaction with three different initial ClO_2 concentrations (20mg/L, 10mg/L, 0.5 mg/L) and reaction times (60min, 30min, 5min). According to the results (Fig.16), it can be said that the influence of the reaction time is not very significant, although a slight decrease of residual ClO_2 concentration with time can be observed for the three different initial tested concentrations. The difference between the lowest (5minutes) and the highest tested reaction time (60 minutes) is around 1mg/L when 10 mg ClO_2/L and 20 mg ClO_2/L are the initial ClO_2 concentrations and this difference is approximately 0.1 mg/L when 0.5 mg ClO_2/L is the initial tested concentration (Fig. 16).

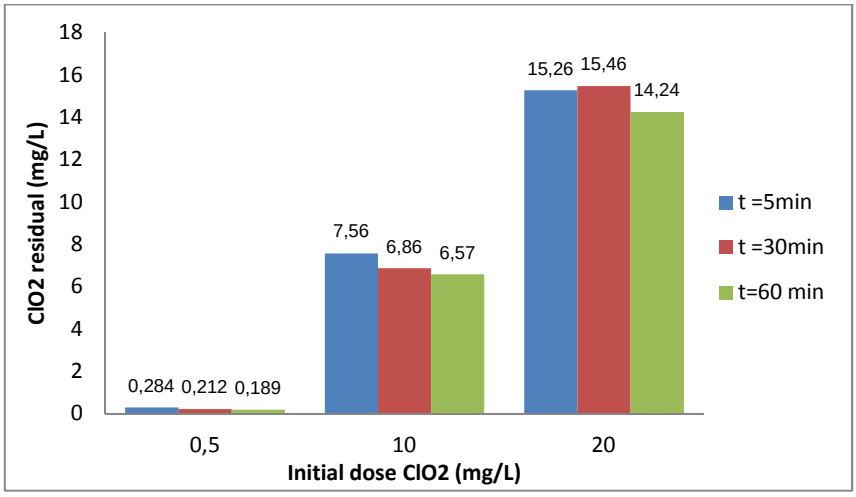

Fig. 16. Influence of reaction time in ClO2 residual concentration.

5.3.2. Influence of COD

In order to study the influence of COD in the residual concentration of ClO_2, two different wastewater effluents with different COD values are tested and seven doses of ClO_2 applied with the same reaction time. A reaction time of 5 minutes was selected for all samples because the result before showed that reaction times higher than 5 minutes do not produce significant variations.

According to the results (Fig. 17a) COD has a clear influence in the residual ClO_2 concentration in wastewater. The effluent with higher COD (60 mg/L) consumes more ClO_2 during the treatment than the effluent with lower COD (21 mg/L). This seems to be logical since organic matter is oxidized by ClO_2 and therefore an effluent with higher COD will demand more oxidant.

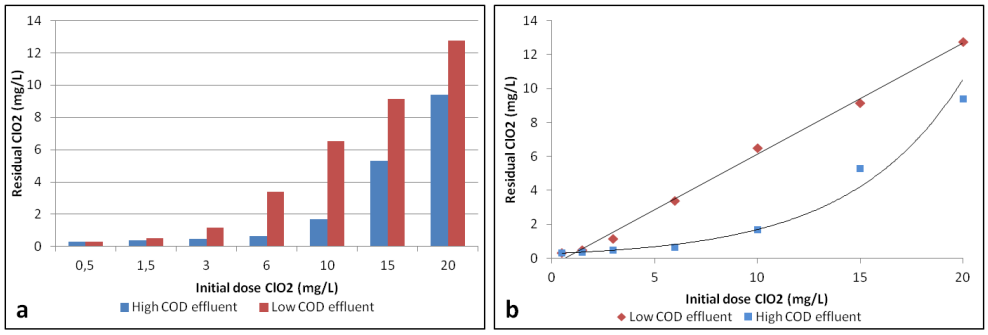

Fig. 17 .Influence of COD in ClO_2 residual concentration in a) bar chart and b) scatter plot. High COD effluent = 60mg/L. Low COD effluent = 21 mg/L.

Results (Fig. 17b) show also that the relationship between initial and residual ClO_2 concentration is linear (R^2 = 0,996) for the low COD effluent; meanwhile for the high COD effluent this relationship turns to be exponential (R^2 = 0,989). When the high COD wastewater effluent is studied, it can be noticed that for initial concentrations of ClO_2 higher than 6 mg/L, there is a faster increase in the ClO_2 residual concentration. Therefore, it could be said that ClO_2 doses higher than 6 mg/L would not be recommended in order to avoid high concentrations of residual ClO_2.

5.4. Relationship between ClO_2 sensitive-absorbance at 254 nm and pharmaceuticals removal

In this section the dose-response of ultraviolet absorbance at 254nm and pharmaceuticals is evaluated at laboratory scale to see if online monitoring of changes in absorbance at 254nm could be used as ClO_2 dosage control.

It can be seen (Fig.18) that after SPE of the water samples, once the analyte has been eluted from the SPE cartridge and dissolved in 1 mL of 0,1wt% EDTA-Na_2 solution, higher doses of ClO_2 lead to a decrease in the coloration of the samples probably due to a decrease of dissolved organic matter (DOC) in the wastewater solution. The sample that has not be treated with ClO_2 (test tube at the right side of the figure) has darker color compared with the other samples. This difference in the color of the samples is especially clear when the no treated sample is compared to the sample treated with 15 mg ClO_2/L (at the left side of the figure). Thus this indicates that the dosage of ClO_2 leads to a change in the coloration of the samples that may correlated with a decrease in COD.

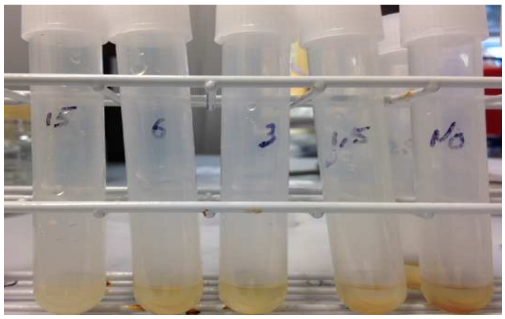

Fig. 18. Color variance after ClO_2 treatment and after SPE (from left to right, sample treated with 15mgClO2/L to a decreasing dose of 1.5 mgClO2/L. Last sample to the right represents the untreated sample).

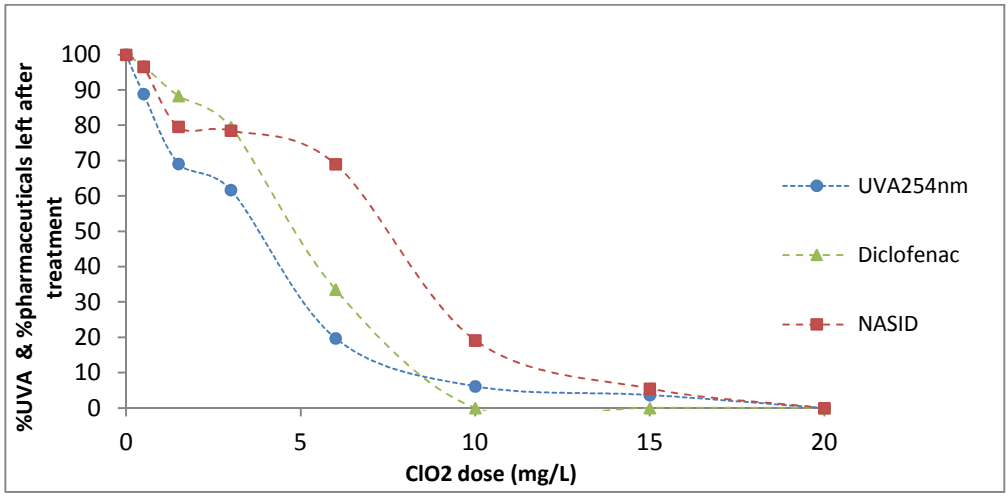

Fig. 19. Dose- response of ClO_2 - sensitive UVA254nm and pharmaceuticals after treatment with seven different doses of ClO_2.

In order to quantitatively verify this relationship, measurement of the absorbance at 254 nm (Fig. 19) shows how a possible dosage control of ClO_2 could be. In order to study the relationship between the ClO_2-sensitive UVA254nm and reduction of pharmaceuticals, removal percentages have been normalized. This means that %UVA left is based on the largest reduction of UVA (62.79%) at the highest dose of 20 mg ClO_2/L (Appendix II- Table 5). The same normalization process was carried out for the studied pharmaceuticals accordingly to their reduction with each dose of ClO_2 (Appendix II – Table 4). The dose – response decay of UVA and diclofenac, which is one of the pharmaceuticals with high environmental risk and recently included in the "watch list" of priority substances, are quite similar (Fig. 19). Both UVA and diclofenac dose-response curves have the fastest decay when the ClO_2 dose has values above 3 mg/L, although their normalized percentages of removal differs almost 20% at this dosage value. However both dose- response curves start to be flat at 10 mg ClO_2/L. The best correlation between the dose –response decay curves of ClO_2 – sensitive UVA and reduction of diclofenac (Fig. 20a) is achieved with a logarithmic fitting line (R^2 =0,992).

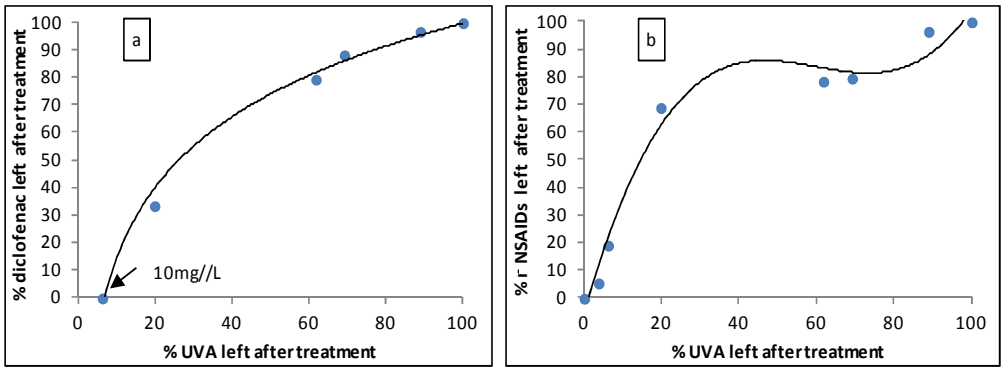

Fig. 20a. Relationship between %ClO_2 - sensitive UVA and %diclofenac left after treatment; b. Relationship between %ClO_2 - sensitive UVA and %NSAIDs left after treatment.

The dose – response decay curve of nonsteroidal anti-inflammatory drugs (NSAIDs), which is an important group of pharmaceuticals due to their high application level worldwide, is calculated as an average normalized value for the removal of diclofenac, ketoprofen, ibuprofen and naproxen (Appendix II- Table 5). The fastest decrease of the NSAIDs decay curve starts in 6 mg ClO_2/L, meanwhile this value was lower (3 mg ClO_2/L) for the ClO_2 sensitive-UVA decay curve. This difference can probably be due to the fact that there are two substances in the NSAIDs group, ibuprofen and ketoprofen, which cannot be completely oxidized by ClO_2. The correlation between ClO_2 sensitive-UVA and reduction of NSAIDs (Fig. 20b) is not logarithmic as for diclofenac but it can be adjusted to a cubic polynomial (R^2= 0,979).

6. DISCUSSION

In this section different aspects of the study are discussed starting by the methodology and possible analytical errors, followed by the optimal ClO_2 dose – time and control strategy retrieved from this study. This section is concluded with a final evaluation of ClO_2 treatment.

6.1. Methodology and analytical errors

The method chosen to get the optimal ClO_2 dose – time as well as possible analytical errors during quantification of pharmaceuticals are addressed in this section.

6.1.1. Factorial experiment and MODDE

The factorial experiment designed in order to find the most optimal ClO_2 dose – reaction time –response has successfully being applied in this study since it has considerably decreased the number of samples to cover a broad range of doses and reaction times. Besides, the combination of the factorial design for oxidation tests together with the utilization of the software tool MODDE has allowed finding the optimal dose – time values in a methodical way to solve a complex optimization. The optimization process in this study has been a big challenge due to the fact that high diversity pharmaceuticals (mode of action, chemical structure, K_{OW}) have been analyzed. Maximum removals for each pharmaceutical were achieved with different ClO_2 dose and reaction time values and therefore MODDE has helped to integrate them and calculate a common theoretical optimal dose-time choosing that result with the most equal factor contribution from both ClO_2 dose and time. In this case an equilibrated contribution of time and ClO_2 consumption has been sought since both can equally contribute for a resource efficient process.

6.1.2. Matrix effects in the quantification of pharmaceuticals

Analytical errors during quantification of pharmaceuticals can occur due to matrix effects caused by the co-elution of sample impurities. These matrix effects such as ion suppression or ion enhancement of the analyte of interest may mask the true concentration of the target analyte in the sample. Thus in order to compensate for the background interferences, blank and standards solutions were utilized. Another approach that has been used for the standardization and calibration of the chromatographic method is the use of internal standards. Isotopic labeled standards, Carbamazepine-13C15N (2µg/mL) and Ibuprofen – D3 (2µg/mL), were used in this study because they are able to compensate for matrix effects and recovery losses of the target analyte in complex matrixes as wastewater is. However, due to the limit availability of proper isotopic

labeled standards, external calibration instead of internal calibration is applied in this study in order to perform the quantification of pharmaceuticals.

Another issue that can affect the analytical method is that despite of the fact that a high diversity of pharmaceuticals with different polarity, molecular shape and size, log K_{OW}, etc. were analyzed, same SPE cartridge and extraction method is utilized. Thus, it is expected that the method and SPE cartridge will work better for some pharmaceuticals and worse for others and therefore different recoveries values varying from 30% to 100% (Appendix I) were obtained for the studied group of pharmaceuticals.

The fact that the same wastewater has been used and frozen until its usage for the experiments has ensured keeping constant background characteristics of the wastewater matrix during all the experiments. This allows eliminating uncertainties when comparing different batches of experiments. Another strategy to avoid background interferences and matrix effects (ion suppression, ion enhancement) from other compounds in wastewater is to study the pharmaceuticals oxidation by ClO_2 in spiked tap water.

6.2. Priority list of pharmaceuticals

The list of prioritized pharmaceuticals was created in order to know which of the analyzed substances in the water are more relevant according to their impact in the ecosystem health downstream of the wastewater treatment plant. This is quite advantageous in the sense that allows focusing on those substances that pose a higher risk for the environment against those that pose lower environmental risk.

When the priority list of pharmaceuticals retrieved from this study (Table 5) is compared with the investigation performed by Sehlén et al. (2015) in Linköping WWTP, oxazepam and metoprolol were also classified as substances with high environmental risk. On the other hand, Shelén et al. (2015) classified diclofenac as a substance with moderate environmental risk and not with a high environmental risk as this study does. The reason for this could be due to the fact that in the investigation carried out by Sehlén et al. (2015) the dilution factor was almost three times higher (27) compared to this study (10) and thus higher PNEC values were considered for the environmental risk ration (EC/PNEC).

The fact that there are not yet any specific guidelines for maximum discharges of these substances to the environment, makes difficult to predict which concentrations levels are acceptable or not. This seems the reason why most of previous studies have focused on removal percentages instead of final concentrations at the effluent of WWTPs. However, this study has given a step forward by taking into account the impact in the receiving water (PNEC) as well as clustering the analyzed substances in different environmental risk groups by comparing EC and PNEC values.

There are different NOEC and assessment factor values that can be applied to calculate PNEC and this fact would then influence the calculated environmental risk ratio (EC/PNEC). However in this study the most sensitive tests, retrieved from FASS, have been selected.

This approach of comparing EC with PNEC, called single substance ecotoxicology effect study, has the disadvantage of assessing effects of the substances individually so it does not take into account combined effects of a mixture of substances whose toxic effect can be larger than

the substance alone. However, this method is still used as a simple way to assess the risk of toxicological effects in the ecosystem.

6.3. Optimal ClO_2 dose – time –response

The final obtained optimal dose – time –response of 5 mg ClO_2/L and 10 minutes can successfully remove (> 90%) more than a half of the analyzed pharmaceuticals which are the most common pharmaceuticals that can be found nowadays at effluent wastewaters.

When the removal efficiencies of each pharmaceutical retrieved from this study (Fig. 14) are compared with previous studies, it can be seen that effectively those substances with electron rich functional groups such as anline (e.g. diclofenac), tertiary amine (e.g. citalopram and ranitidine), secondary amine (e.g. propanolol) or sulfonamide (e.g. furosemide) can be oxidized by ClO_2. On the other hand, those pharmaceuticals with non-electron rich functional groups such as carbamazepine, ketoprofen and the β- blocker atenolol have poor removal with ClO_2 as stated by Hey et al. (2012) and Lee and von Gunten (2010).

However, some disagreements have been found in the removal of β-blockers metoprolol, bisoprolol and the anti depressant fluoxetine when comparing this study with Hey et al. (2012). In this study these three pharmaceuticals have maximum removal with the optimal ClO_2 dose – reaction time, meanwhile Hey et al. (2012) states that these substances cannot be completely oxidized even with the highest dose of 20 mg ClO_2/L. In order to study the disagreement between this study and Hey et al. (2012), oxidation tests were carried out in spiked tap water in order to avoid possible interferences produced by the wastewater matrix such as ion suppression. After this test, metoprolol, bisoprolol and fluoxetine still had a maximum removal with the optimal ClO_2 dose – reaction time what should indicate that they can be successfully degraded by ClO_2.

Accordingly to the priority list of pharmaceuticals, six of eight pharmaceuticals that had high and moderate environmental risk before treatment, can be classified as substances that pose a low risk for the environment after treatment with optimal ClO_2 dose – reaction time. However, the main concern lies on oxazepam which is a common sedative that pose a high risk for the ecosystem health even after treatment with ClO_2 since it can only be removed around 30% with ClO_2. Other oxidation technologies such as ozone can neither completely remove oxazepam although its removal (80% removal with 7 g O_3/m^3) is much higher than with ClO_2. However, oxazepam can be almost totally removed (>99%) with activated carbon (Baresel et al. 2014).

It could be said that the optimal ClO_2 dose –time (5 g ClO_2/m^3 - 10 minutes) retrieved from this study is coherent and could be applied in future upscaling. This is because when this optimal dose–time is compared with previous experiments with ozone, similar dose (7 g O_3/m^3) and retention time (10 minutes) were obtained giving a high removal (>94%) for all studied pharmaceuticals except oxazepam (Baresel et al., 2014).

Two factorial experiments have been carried out in this study in order to constrain the dose and time ranges and get an optimal ClO_3 dose-time as accurate as possible. Even though the optimal dose-time value seems quite reasonable, both for ClO_2 dose and reaction time, one more factorial experiment could be done in order to narrow the ClO_2 dose range and see if it is feasible to obtain even lower optimal ClO_2 dose.

6.4. ClO_2 dosage control

To find a proper optimal dose – time for pharmaceuticals removal using ClO_2 can be considered as the first step in order to see if it is possible to apply this technology in a resource – efficient way oxidizing most relevant pharmaceuticals. However, for future full scale implementation of this technology, it is important to take into account that composition of wastewater varies over time, especially in small scale wastewater treatments (< 2000 pe) where incoming flows and loads changes widely in comparison to major treatment plants (Rivera, 2006). In this case, it is not so relevant to have one optimum dose-time but to adapt the dosage according to the load variations in order to avoid overdose and an increase risk of formation of eco-toxic byproducts. Results clearly showed that variations in COD affect the residual ClO_2 concentration. For instance, for the optimal dose of 5 mg ClO_2/L the residual ClO_2 is about 13% and 50% of the initial oxidant concentration for the high COD and low COD wastewater effluents respectively after 5 minutes of reaction. Furthermore, pharmaceuticals removal would differ in wastewaters with different COD values since pharmaceuticals would compete against the oxidant demand of aromatic compounds. Therefore, measuring the decrease of ultraviolet absorbance at 254 nm, which is a rough measure of aromatic rings in the water, can be a promising control strategy and easy to implement on high quality effluents free of suspended particles.

Results showed a clear dose-response relationship, especially between UVA254nm and diclofenac. However, further research should be performed since different wastewater matrices will have specific absorbance profiles. Also real online monitoring of absorbance at 254 nm could be performed at the effluent and water samples taken at specific times to measure pharmaceuticals concentration in order to correlate it with the absorbance at 254 nm.

6.5. Final evaluation

Three different factors can be discussed to evaluate the usefulness of ClO_2 as end of pipe treatment for the effluent of WWTPs: efficiency of the treatment, cost and risk of formation of ecotoxicological byproducts.

6.5.1. Efficiency of ClO_2 treatment

According to the efficiency of the treatment, ClO_2 could be considered a suitable technology to remove pharmaceuticals from wastewater since more than a half of the 17 studied pharmaceuticals can be removed (>90%) and six of eight pharmaceuticals which had a significant environmental risk have a low risk after treatment with the optimal ClO_2 dose – time (5g ClO_2/m^3 – 10minutes).

However, the fact that other technologies (e.g. ozone, activate carbon) have higher removal efficiencies for those substances that cannot be completely removed by ClO_2 (e.g. oxazepam, carbamazepine) and that ozone reacts faster with the same functional group resulting in shorter half-lives than ClO_2 (Huber et al. 2005), can relegate the use of ClO_2 as end of pipe treatment for the effluent of WWTPs.

One approach could be based on combining ClO_2 treatment with activate carbon as post-treatment so the deficiencies of one technology can be solved by the other. Other studies have already tested combined systems at pilot scale (ozone + activate carbon) obtaining quite satisfactory results (Baresel et al. 2015). However, this combined systems could be only feasible in conventional WWTPs but not is small scale

WWTPs (<2000 pe) since they do not usually apply advanced treatment technologies in their wastewater facilities.

6.5.2. Cost

According to the cost, it is known that the capital cost of other oxidation technologies such as ozone (100-300 k€) is much higher than a ClO_2 generator (10-30 k€). On the other hand, ozone has a very competitive running cost of about 0,6 SEK/ m^3 (Baresel et al., 2015) meanwhile running cost for the generation of ClO_2 are usually higher since it needs to be produced on site from $NaClO_2$ solution. However, new studies have tested the possibility to produce ClO_2 by electrocatalytical process with both NaCl and $NaClO_2$ that would reduce production cost (Tsai et al., 2014). Furthermore, the fact that the optimal ClO_2 dose – reaction time (5 g ClO_2/m^3 – 10 minutes) retrieved from this study and the dose – retention time tested with ozone (7 g O_3/m^3 – 10 minutes) are quite similar, could mean that ClO_2 treatment could be economically competent against other technologies.

If the efficiency of the treatment and cost are evaluated together it can be said that ClO_2 could definitely be satisfactorily applied as end of pipe treatment is small scale WWTP where the application of the optimal ClO_2 dose – time would eliminate a high diversity of pharmaceuticals with a lower initial cost compared to ozone.

However one factor that would definitively support the choice of an oxidation technology as the most suitable end of pipe treatment for pharmaceuticals removal is to discard the risk of formation of transformation byproducts than can be more toxic than the parent compound.

6.5.3. Ecotoxicological by-products

It is known that the main drawback of oxidation technologies is the possibility of formation of unwanted substances that can be even more toxic than the parent compounds. According to previous studies about disinfection of drinking water with ClO_2, the main risk belongs to the formation of inorganic by-products although some organic by-products have also been reported. However, formation of by-products after pharmaceuticals removal has not been extensively reported.

In this study the toxicity of few samples was retrieved from microtox test. After this test the treated sample with ClO_2 had a $EC_{50,15min}$ value (11.37%) much higher compared to an untreated wastewater sample that turned to be no toxic ($EC_{50,15min}$ > 100%). In order to try to decrease this toxicity, a post treatment step (theoretical dose of 4.14 mg Fe^{2+}/mg ClO_2) followed by sand filter) was applied at laboratory scale after ClO_2 dosage in order to remove inorganic byproducts and residual ClO_2 since previous studies (Sorlini & Collivignarelli, 2005; Katz & Narkis, 2001) have stated that it is possible to remove them by this technique. However, the microtox test performed with this sample still gave an $EC_{50, 15min}$ toxicity value (15.8%) higher than the untreated sample and a similar value compared to the treated sample with ClO_2. This could be due to the fact that ClO_2 and inorganic byproducts were still left in solution after treatment with the iron salt or due to the presence of organic compounds that cannot be reduced by the iron salt. The presence of organic byproducts after ClO_2 treatment could be feasible since a recent investigation performed by Wang et al. (2014), that studied the major disinfection by-products after oxidation of diclofenac by ClO_2, reported that during degradation process intermediate substances such as

hydroxyl-compounds (Fig. 21) more toxic than the parent compound diclofenac were formed.

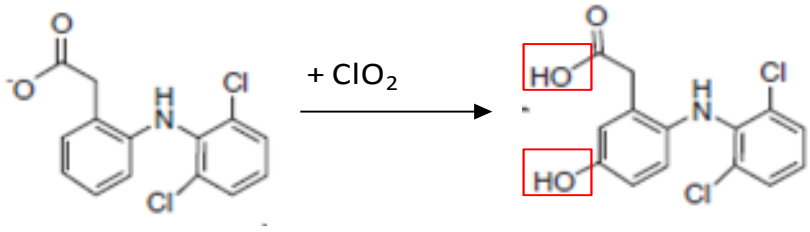

Fig. 21. Hydroxylation of diclofenac with ClO_2.

In order to know if there were inorganic byproducts or ClO_2 left after the reaction with the iron salt, the iron treated sample was filtered through activate carbon to adsorb the possible organic byproducts in solution formed after ClO_2 treatment. $EC_{50,\ 15min}$ toxicity after this treatment was still high (13.61%) what could mean that some inorganic substances still remained after iron dosage or due to other compounds that cannot be retained neither by sandfilter nor with activate carbon (e.g. free hydroxyl radicals).

It was not possible to draw conclusive findings from these microtox tests since few experiments were performed and the iron salt addition was not optimized. However, one factor that could have a large influence in the experiments and that have not been evaluated in this study is the retention time between addition of the iron salt to the ClO_2 treated sample and filtration through the sand filter. This may be an important parameter to be taken into account since free hydroxyl radicals (-OH) formed after oxidation, which are known to be very toxic and that cannot be retained neither by sandfilter nor by activate carbon, may not have had time to react with organic matter in solution and therefore they could have increased the toxicity of the water solution. If these free –OH radicals would have reacted with organic matter, they would have formed new organic hydroxyl-compounds that are, in most of the cases, less toxic and more biodegradable than the parent compound. Besides they can be retained by conventional systems (e.g. sandfilter). There are however some cases, such as in the oxidation of diclofenac, where the intermediate hydroxyl compounds (Fig. 21) are more toxic than the parent compound but in this case these substances can be removed by activate carbon. Finally, it should be noted that the formation of free –OH radicals is an inherent and common problem with other oxidative agents such as treatment with ozone, H_2O_2 or photolytic degradation and not only for ClO_2 treatment.

It can be said that further research and more tests should be carried out concerning the toxicity and formation of byproducts after reaction with ClO_2 and also compare it with other oxidation technologies (e.g. ozone, peroxides, photolytic degradation) that may have similar problem with free –OH radicals.

7. FUTURE STUDIES

The result of the bench-scale study shows that ClO_2 is effective removing most of the studied pharmaceuticals when the optimal dose - time (5g ClO_2/m^3 – 10 minutes) is applied. However, further work should be done in the following areas:

Deeper research needs to be done in the study of ecotoxicological byproducts generated after the treatment with ClO_2 before upscaling this technology to pilot or full scale WWTPs. More tests with the post treatment step (ferrous salt followed by sand filter) to remove inorganic byproducts and subsequent evaluation of toxicity with microtox tests should be performed. However retention time between addition of the ferrous salt and sand filtration should be evaluated in order to determine if intermediate products such as free –OH radicals, more toxic than the parent compounds, can be degraded over time by reaction with organic matter before going through the sand filter. It would also be of great value to identify degradation products after ClO_2 treatment by analyzing the peaks from HPLC –MS/MS chromatogram.

As the optimal ClO_2 dose – time for maximum pharmaceuticals removal has already been tested and retrieved from this study, the next step should be more focus on optimizing the control dosage strategy of ClO_2 accordingly to hourly variations in the load and composition of the wastewater matrix. This would be valuable in order to achieve a resource efficient process and to avoid overdosing of the oxidant for a future upscale application.

The list of prioritized substances presented in this study has only included the most relevant pharmaceuticals that can be found nowadays at WWTPs effluents. However this is a dynamic list that can be modified if other micropollutants, which have been reported in wastewater effluents, were included. Some relevant micropollutants whose sensitivity to be oxidized by ClO_2 could be evaluated are: i) phthalates esters which are commonly used as plasticizers in different applications such as wall covering or medical applications; ii) flame retardants such as polybrominated diphenyl ethers (PBDEs) and phosphorous flame retardants (PFRs); iii) per- and polyfluoroalkyl substances (PFAS); iv) phenolic substances such as BPA and v) multi resistance bacteria formed by the increased use of antibiotics.

8. CONCLUSION

The results from this bench scale study shows that an optimal dose of 5 g ClO_2/m^3 and a reaction time of 10 minutes can totally remove more than a half of the studied pharmaceuticals. Furthermore six of the eight pharmaceuticals that posed moderate and high environmental risk before ClO_2 treatment, had a low environmental risk after treatment with ClO_2. Only oxazepam and carbamazepine keep high and moderate environmental risk respectively after ClO_2 treatment. Results indicate also that variations in wastewater composition (e.g. COD) would affect the oxidant demand and therefore ClO_2 – sensitive absorbance at 254nm and pharmaceuticals dose-response decay curves were measured as possible strategy for ClO_2 dosage control.

However, before stating if ClO_2 could be a suitable end of pipe treatment for WWTPs, deeper research and evaluation of ecotoxicological byproducts needs to be performed.

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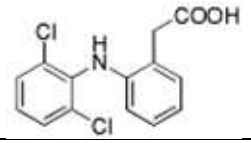
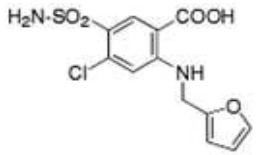
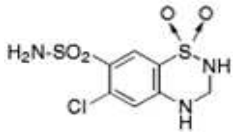
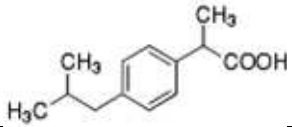
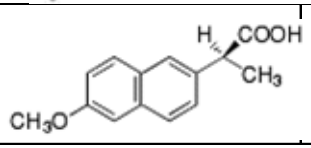
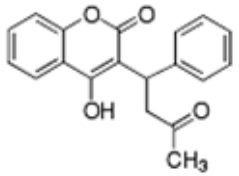
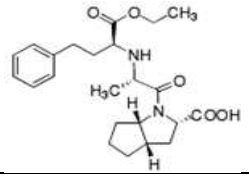
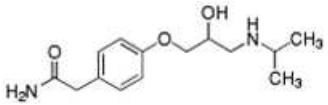
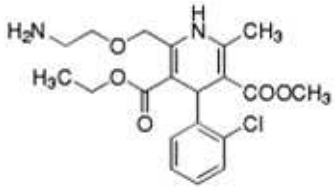
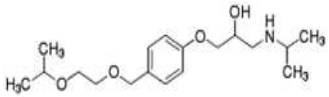
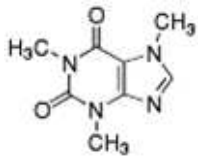
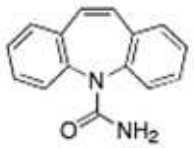
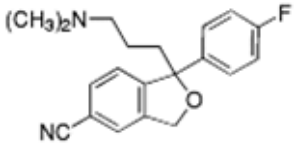
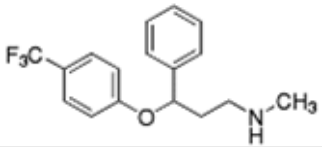
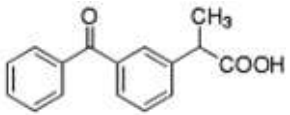
APPENDIX I - CHARACTERISTICS OF 23 ANALYZED PHARMACEUTICALS

Substance	Mode of action	Chemical structure	Ion Mode	Retention time (min)	Recovery %	LOD (ng/L)	LOQ (ng/L)
Diclofenac	*Anti-inflammatories*		Negative	12,4	75,40	4,2	14,13
Furosemide	*Diuretics*		Negative	9,28	30,63	6,8	22,67
Hydrochlorothiazide	*Antihypertensives*		Negative	5,07	37,32	3,2	10,67
Ibuprofen	*Anti-inflammatories*		Negative	12,5	33,52	3,5	11,52
Naproxen	*Anti-inflammatories*		Negative	11,3	52,02	5,1	16,88

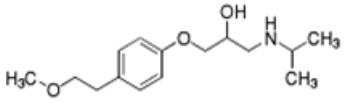
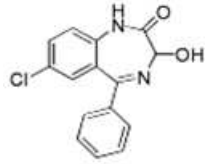
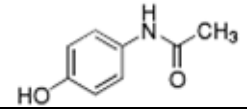
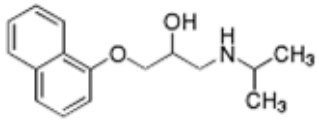
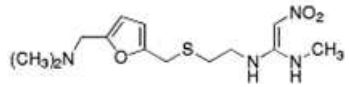
**Recovery, LOD and LOQ calculated with triplicate samples*

Substance	Mode of action	Chemical structure	Ion Mode	Retention time (min)	Recovery %	LOD (ng/L)	LOQ (ng/L)
Ramipril	*Antihypertensives*		Negative	10,8	85,24	2,6	8,82
Warfarin	*Anticoagulants*		Negative	11,4	146,67	2,2	7,45
Atenolol	*Antihypertensives*		Positive	4,56	81,70	4,5	15,03
Amlodipine	*Antihypertensives*		Positive	8,91	29,01	1,8	6,03
Bisoprolol	*Antihypertensives*		Positive	7,51	114,64	1,3	4,25

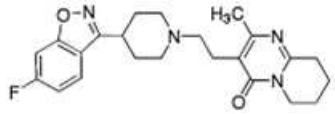
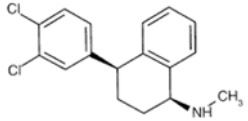
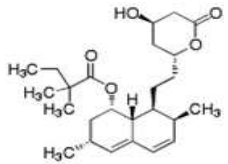
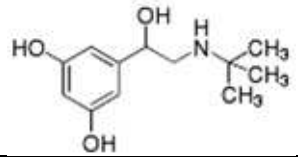
**Recovery, LOD and LOQ calculated with triplicate samples*

Substance	Mode of action	Chemical structure	Ion Mode	Retention time (min)	Recovery %	LOD (ng/L)	LOQ (ng/L)
Caffein	*Stimulant*		Positive	6,08	60,0	38,2	127,36
Carbamazepine	*Sedatives*		Positive	9,02	82,64	17,6	58,71
Citalopram	*Antidepressants*		Positive	7,87	89,97	2,0	6,63
Fluoxetine	*Antidepressants*		Positive	8,98	63,49	1,6	5,25
Ketoprofen	*Anti-inflammatories*		Positive	9,75	41,93	4,9	16,19

**Recovery, LOD and LOQ calculated with triplicate samples*

Substance	Mode of action	Chemical structure	Ion Mode	Retention time (min)	Recovery %	LOD (ng/L)	LOQ (ng/L)
Metoprolol	*Antihypertensives*		Positive	6,65	73,3	4,8	16,03
Oxazepam	*Sedatives*		Positive	9,55	73,91	5,2	17,36
Paracetamol	*Anti-inflammatories*		Positive	4,47	51,21	10,5	34,89
Propranolol	*Antihypertensives*		Positive	7,85	90,22	0,8	2,58
Ranitidine	*Antiulcers*		Positive	4,54	82,40	4,0	13,33

**Recovery, LOD and LOQ calculated with triplicate samples*

Substance	Mode of action	Chemical structure	Ion Mode	Retention time (min)	Recovery %	LOD (ng/L)	LOQ (ng/L)
Risperidone	*Antipsychotic*		Positive	7,36	133,86	2,2	7,37
Sertralin	*Antidepressants*		Positive	9,19	51,81	2,0	6,65
Simvastatin	*Lipid-regulating*		Positive	11,6	47,46	1,99	6,66
Terbutaline	*Asthma medication*		Positive	4,44	53,56	2,6	8,80

**Recovery, LOD and LOQ calculated with triplicate samples*

APPENDIX II - RESULTS FROM ANALYSIS OF PHARMACEUTICALS

Table 1. Results from first factorial experiment.

	60C3				30C3				5C3				60C2				30C2			
	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%
Diclof	<14.1	<14.1	<14.1	100	<14.1	<14.1	<14.1	100	<14.1	<14.1	<14.1	100,00	<14.1	<14.1	<14.1	100	<14.1	<14.1	<14.1	100
Furos	<22.7	<22.7	<22.7	100	<22.7	<22.7	<22.7	100	<22.7	<22.7	<22.7	100	27,79	35,22	31,50	98,12	163,99	151,22	157,60	90,58
Hydrochl	453,66	452,24	452,95	58,58	485,77	447,40	466,58	57,34	455,12	449,25	452,18	58,65	594,47	570,36	582,42	46,75	606,01	578,11	592,06	45,86
Ibu	43,0	34,7	38,84	22,50	44,4	45,6	45,02	10,18	35,5	19,6	27,52	29,22	21,2	47,4	34,30	31,56	23,0	10,0	16,50	67,08
Naprox	<16.88	<16.88	<16.88	100	<16.88	<16.88	<16.88	100	9,00	6,59	7,79	98,46	<16.88	<16.88	<16.88	100	0,99	3,12	2,06	99,59
Ateno	239,54	230,43	234,98	5,09	242,37	224,69	233,53	5,68	229,47	242,77	236,12	4,63	260,88	223,41	242,15	2,19	221,13	181,95	201,54	18,59
Amlo	<6,03	<6,03	<6,03	100,00	<6,03	<6,03	<6,03	94,73	<6,03	<6,03	<6,03	94,73	0,27	<6,03	0,27	98,41	3,45	0,13	1,79	89,56
Bisop	55,25	57,39	56,32	13,43	49,59	38,62	44,10	32,21	28,65	55,49	42,07	55,96	54,27	43,94	49,11	24,52	33,17	10,36	21,77	66,54
Carba	231,32	233,13	232,22	6,81	231,60	221,74	226,67	9,04	240,70	237,51	239,10	4,05	254,78	244,76	249,77	0,00	242,94	248,44	245,69	1,40
Citalo	64,425	7,310	35,87	80,93	55,753	1,622	28,69	84,75	0,252	16,277	8,26	95,61	68,317	9,779	39,05	79,24	2,091	<6,63	<6,63	100
Fluox	6,03	6,14	6,08	71,39	1,79	<5,25	<5,25	100,00	<5,25	3,60	3,60	83,05	7,13	2,35	4,74	77,70	<5,25	<5,25	<5,25	100
Keto	281,10	290,57	285,83	2,76	288,12	279,21	283,66	3,50	276,75	281,23	278,99	5,09	290,98	283,99	287,49	2,20	283,90	286,11	285,01	3,04
Meto	942,78	948,73	945,75	7,18	787,48	637,81	712,65	30,06	448,04	946,94	697,49	31,55	959,17	791,37	875,27	14,10	543,95	161,99	543,95	46,61
Oxa	239,78	225,41	232,59	29,18	252,67	224,45	238,56	27,36	261,03	239,72	250,37	23,76	240,26	217,41	228,84	30,32	226,41	237,46	231,94	29,38
Prop	2,17	0,71	1,44	97,69	1,27	<2,58	<2,58	100,00	0,02	3,24	1,63	97,38	27,34	11,24	19,29	69,09	3,77	0,04	1,91	96,95
Rani	1,10	<13,3	<13,3	100,00	0,13	<13,3	<13,3	98,64	<13,3	<13,3	<13,3	98,64	<13,3	<13,3	<13,3	98,64	<13,3	<13,3	<13,3	98,64
Sert	9,22	5,88	7,55	82,96	0,64	<6,65	<6,65	100	<6,65	0,35	0,35	99,21	12,55	0,84	6,69	84,90	<6,65	<6,65	<6,65	97,75

Table 1 (cont.). Results from first factorial experiment.

	5C2				60C1				30C1				5C1				No treatment			
	1 (ng/L)	2 (ng/L)	Final (ng/L)	%	1 (ng/L)	2 (ng/L)	Final (ng/L)	%	1 (ng/L)	2 (ng/L)	Final (ng/L)	%	1 (ng/L)	2 (ng/L)	Final (ng/L)	%	1 (ng/L)	2 (ng/L)	3 (ng/L)	Average (ng/L)
Diclof	<14.1	<14.1	<14.1	100	430,98	329,27	380,13	29,40	329,18	385,75	357,47	33,61	284,79	340,21	312,50	41,96	450,97	576,86	587,44	538,42
Furos	502,01	356,58	429,30	74,34	964,05	897,73	930,89	44,36	1017,13	1168,31	1092,72	34,69	1017,89	1294,83	1156,36	30,89	1408,90	1812,38	1798,33	1673,20
Hydrochl	589,32	584,27	586,79	46,35	1404,37	1616,05	1510,21	0,00	1581,32	1416,81	1499,06	0,00	1296,43	1505,67	1401,05	0,00	931,64	1199,59	1149,71	1093,65
Ibu	11,6	29,4	20,53	59,04	40,1	29,3	34,69	30,78	42,3	29,6	35,92	28,34	29,6	37,6	33,58	33,00	24,26	32,89	50,12	50,12
Naprox	76,53	43,64	60,09	88,14	342,85	343,12	342,98	32,30	363,73	378,94	371,33	26,71	318,44	377,05	347,74	31,36	387,22	561,70	571,04	506,65
Ateno	226,35	243,40	234,88	5,13	259,31	237,83	248,57	0,00	148,87	249,57	199,22	19,53	109,62	73,06	109,62	55,72	181,78	247,58	234,95	247,58
Amlo	0,71	<6,03	0,71	95,84	28,69	4,99	16,84	1,96	13,10	0,17	6,64	61,36	5,32	6,84	6,08	64,62	15,38	18,14	18,01	17,18
Bisop	40,27	21,74	31,01	52,34	52,89	35,97	44,43	31,70	10,12	55,46	32,79	49,60	6,86	0,14	3,50	94,62	59,91	68,42	66,84	65,06
Carba	244,91	236,79	240,85	3,34	258,97	254,74	256,86	0,00	263,62	266,58	265,10	0	199,18	267,00	233,09	6,46	212,54	259,83	275,19	249,19
Citalo	4,292	<6,63	4,29	97,72	34,361	14,390	24,38	87,04	0,295	71,181	35,74	81,00	0,160	<6,63	0,16	99,91	175,08	194,05	195,25	188,13
Fluox	1,01	<5,25	1,01	95,22	14,07	7,73	10,90	48,73	5,73	15,12	10,43	50,95	5,38	7,73	6,55	69,17	20,40	22,39	20,97	21,26
Keto	273,78	268,86	271,32	7,70	285,41	304,14	294,77	0,00	304,75	296,68	300,72	0	246,86	308,09	277,48	5,60	228,41	322,45	331,00	293,95
Meto	645,07	355,10	500,09	50,92	888,06	571,38	729,72	28,38	147,76	913,53	530,64	47,92	107,28		107,28	89,47	903,79	1089,09	1063,89	1018,92
Oxa	244,18	234,60	239,39	27,11	294,22	303,02	298,62	9,07	309,28	314,81	312,04	4,99	234,35	321,17	277,76	15,43	292,59	348,24	344,43	328,42
Prop	5,61	<2,58	5,61	91,01	25,87	7,14	16,51	73,55	0,43	29,26	14,85	76,21	0,26	<2,58	0,26	99,58	58,01	66,19	63,03	62,41
Rani	<13,3	<13,3	<13,3	98,64	0,87	1,93	1,40	99,05	<13,3	3,07	3,07	97,91	<13,3	<13,3	<13,3	98,64	128,09	183,15	129,75	147,00
Sert	<6,65	<6,65	<6,65	97,75	2,75	0,04	1,40	96,85	<6,65	6,53	6,53	85,27	<6,65	<6,65	<6,65	97,75	42,02	43,93	46,98	44,31

Table 2. Results from second factorial experiment.

	60C3		30C3		5C3		60C2		30C2		5C2		60C1		30C1		5C1		No treated (ng/L)
	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	C (ng/L)	%	
Diclof	<13.01	100	<13.01	100	<13.01	100	<13.01	100,00	<13.01	100	<13.01	100	<13.01	100,00	<13.01	100,00	<13.01	100,00	772,90
Furos	<10,61	100	5,52	99,38	29,88	96,64	<10,61	100,00	75,42	91,51	178,38	79,93	291,10	67,24	340,24	61,71	173,58	80,47	888,7
Hydrochl	251,68	71,46	438,45	50,28	384,07	56,44	335,20	61,99	490,60	44,36	476,19	46,00	915,01	0,00	834,94	5,31	1964,51	0,00	881,77
Naprox	<11,36	100,00	<11,36	100,00	<11,36	100,00	<11,36	100,00	<11,36	100	31,68	95,08	57,32	91,10	134,45	79,14	37,96	94,11	644,41
Ateno	154,21	28,39	242,73	0,00	198,10	8,01	132,60	38,43	187,11	13,11	192,32	10,69	222,82	0,00	171,35	20,43	261,46	0,00	215,34
Amlo	<7,48	100,00	<7,48	100,00	<7,48	100,00	<7,48	100,00	<7,48	100,00	<7,48	100,00	<7,48	100,00	<7,48	100,00	<7,48	100,00	18,40
Bisop	<2,07	100,00	0,67	98,94	0,80	98,73	<2,07	100,00	<2,07	100	0,00	100,00	0,21	99,66	<2,07	100,00	3,24	94,85	62,91
Carba	243,32	24,13	354,91	0,00	227,89	28,94	56,26	82,46	253,23	21,04	195,01	39,19	387,37	0,00	361,14	0	272,76	14,95	320,71
Citalo	<3,89	100,00	<3,89	100,00	0,08	99,96	<3,89	100,00	<3,89	100	<3,89	100,00	<3,89	100,00	<3,89	100,00	<3,89	100,00	214,76
Fluox	<7,12	100,00	<7,12	100,00	<7,12	100,00	<7,12	100,00	<7,12	100,00	<7,12	100,00	<7,12	100,00	<7,12	100,00	<7,12	100,00	15,10
Keto	332,41	0,00	351,74	0,00	218,36	30,98	61,39	80,60	221,16	30,10	162,75	48,56	361,43	0,00	324,03	0	343,22	0,00	316,38
Meto	<12,3	100,00	22,85	97,70	25,20	97,46	1,17	99,88	0,36	99,96	4,34	99,56	7,50	99,24	<12,3	100,00	21,54	97,83	993,07
Oxa	253,29	33,11	379,21	0,00	122,46	67,66	38,84	89,74	187,28	50,54	148,65	60,74	422,17	0,00	387,44	0	327,45	13,52	378,65
Prop	<3,01	100,00	<3,01	100,00	<3,01	100,00	<3,01	100,00	<3,01	100	<3,01	100	<3,01	100,00	<3,01	100	2,34	95,98	58,02
Sert	<3,37	100,00	<3,37	100,00	<3,37	100,00	<3,37	100,00	<3,37	100	<3,37	100	<3,37	100,00	<3,37	100	5,85	84,97	38,88

***C3 = 15mg/L ClO_2; C2 = 10 mg/L ClO_2; C1 = 4 mg/L ClO_2*

Table 3. Results from verification of the two optimums (Duplicate samples).

	Optimum 1 (10mg/L - 5min)				Optimum 2 (5mg/L - 10min)				No treated (ng/L)
Pharmaceutical	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	% removal	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	% removal	
Diclof	<14,13	<14,13	<14,13	100,00	<14,13	<14,13	<14,13	100,00	597,04
Bisop	<4,25	<4,25	<4,25	100,00	<4,25	<4,25	<4,25	100	64,52
Meto	<16,03	<16,03	<16,03	100,00	<16,03	<16,03	<16,03	100	1012,46
Citalo	<6,63	<6,63	<6,63	100,00	<6,63	<6,63	<6,63	100,00	194,79
Rani	<13,33	<13,33	<13,33	100,00	<13,33	<13,33	<13,33	100,00	147,00
Sert	<6,65	<6,65	<6,65	100,00	<6,65	<6,65	<6,65	100,00	42,95
Fluox	<5,25	<5,25	<5,25	95,22	<5,25	<5,25	<5,25	95,00	19,71
Prop	<2,58	<2,58	<2,58	95,00	<2,58	<2,58	<2,58	95,00	61,31
Amlo	<6,03	<6,03	<6,03	95,84	<6,03	<6,03	<6,03	95	16,74
Naprox	53,02	62,73	57,88	88,14	101,58	110,62	106,10	80,39	541,09
Furos	402,47	302,53	352,50	74,34	540,49	584,74	562,61	61,91	1477,07
Ibu	20,61	15,34	17,975	50,00	23,8	30,8	27,29	23,69	35,76
Hydrochl	535,92	497,07	516,50	46,35	791,23	750,46	770,85	25,93	1040,68
Oxa	215,95	230,53	223,24	34,53	230,83	207,19	219,01	30,00	340,98
Keto	271,86	343,17	307,52	10,06	317,45	289,89	303,67	8,26	331,00
Carba	236,37	291,38	263,88	7,54	253,26	245,42	249,34	6,64	267,07
Ateno	191,34	200,35	195,84	6,04	185,66	228,96	207,31	5,73	219,91

Table 4. Results from seven doses of ClO2 for NSAIDs.

	3 mg/L				1,5 mg/L				0,5 mg/L				No treated mg/L		
	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)
Diclof		521,12	521,12	20,52	577,87	580,39	579,13	11,67	568,32	701,21	634,77	3,18	590,84	720,41	655,62
Ibu		1643	1181,07	34,78	871,44	1658	1264,72	30,16	858,33	2611	1734,74	4,21	1379,73	2242	1810,96
Keto		490,34	490,34	0,00	462,73	500,73	481,73	0,00	399,27	509,97	454,62	0,00	380,63	467,24	423,93
Naprox		293,62	293,62	3,58	255,78	261,31	258,54	15,10	263,69	328,18	295,93	2,82	275,59	333,44	304,51

	20 mg/L				15 mg/L				10 mg/L				6 mg/L			
	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%	C1 (ng/L)	C2 (ng/L)	Average (ng/L)	%
Diclof	<7,69	<7,69	<7,69	100,00	<7,69	<7,69	<7,69	100	<7,69	<7,69	<7,69	100,00	298,99	140,53	219,76	66,48
Ibu	868,92	701,78	785,36	56,63	329,63	1287	808,33	55,36	495,40	1360	927,58	48,78	869,129	2003	1435,98	20,71
Keto	345,96	378,51	362,23	45,00	93,01	389,27	241,14	43,12	174,86	463,78	319,32	24,68	432,87	496,60	464,74	0,00
Naprox	9,84	46,07	27,96	90,82	14,80	74,57	44,69	85,33	61,73		61,73	79,73	247,86	245,60	246,73	18,98

Table 5. Normalized percentages for NSAIDs and UVA left after treatment.

	NSAIDs					UVA 254 nm	
ClO2 dose (mg/L)	Diclof (% left)	Ibu ((% left)	Keto (% left)	Naprox ((% left)	Average NSAIDs (%)	Absorbance	% left
20	0,00	0,00	0,00	0,00	0,00	0,048	0,00
15	0,00	2,24	13,76	6,05	5,51	0,051	3,70
10	0,00	13,87	50,65	12,21	19,18	0,053	6,17
6	33,52	63,44	100,00	79,11	69,02	0,064	19,75
3	79,48	38,58	100,00	96,06	78,53	0,098	61,73
1,5	88,33	46,74	100,00	83,38	79,61	0,104	69,14
0,5	96,82	92,57	100,00	96,90	96,57	0,12	88,89
0	100,00	100,00	100,00	100,00	100,00	0,129	100,00

APPENDIX III - OPTIMAL CLO_2 DOSE –TIME-RESPONSE FOR EACH PHARMACEUTICAL

First factorial experiment			
Pharmaceutical	C	t	% removal
Diclof	10	5	99
Bisop	10	5	99
Meto	8	5	99
Citalo	12,2	43,5	99
Furos	10	30	99
Rani	0,62	7	95
Sert	1	12	95
Fluox	10	5	95
Prop	14,15	32,5	95
Amlo	10	5	94
Naprox	12,2	43,5	93
Hydrochl	18	30	60
Ibu	10	16	49
Oxa	10	54	30
Ateno	10	30	20
Carba	10	60	10
Keto	10	60	8

Second factorial experiment			
Pharmaceutical	C	t	% removal
Diclof	4	5	99
Sert	4	5	99
Citalo	4	5	99
Meto	4	5	97
Rani	4	5	95
Bisop	4	5	95
Amlo	4	5	95
Fluox	10	5	95
Prop	4	5	95
Naprox	7	38	95
Furos	11,7	32	95
Hydrochl	15	60	70
Ibu	10	16	49
Oxa	10	54	30
Ateno	10	30	20
Carba	10	60	10
Keto	10	60	8