CENTRO DE INVESTIGACIÓN Y ASISTENCIA EN TECNOLOGÍA Y DISEÑO DEL ESTADO DE JALISCO, A. C.

COMPUESTOS EMERGENTES: OPTIMIZACIÓN DE MÉTODOS ANALÍTICOS PARA LA DETERMINACIÓN EN MUESTRAS DE AGUA Y EVALUACIÓN DE CINÉTICA DE OXIDACIÓN USANDO OZONO

TESIS

QUE PARA OBTENER EL GRADO
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EN LA ESPECIALIDAD DE

INGENIERÍA AMBIENTAL

PRESENTA

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CIENCIA Y TECNOLOGÍA

CENTRO DE INVESTIGACIÓN Y ASISTENCIA EN TECNOLOGÍA Y DISEÑO DEL ESTADO DE JALISCO, A. C.

EMERGING COMPOUNDS: OPTIMIZATION OF ANALYTICAL METHODS FOR DETERMINING IN WATER SAMPLES AND ASSESSMENT OF OXIDATION KINETICS USING OZONE

THESIS

to obtain the degree of

DOCTOR IN SCIENCE AND TECHNOLOGY

Presented by:
M.D. RAMIRO VALLEJO RODRÍGUEZ

Directed by:
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Guadalajara City, Jal., septiembre 2012.

CIENCIA Y TECNOLOGÍA

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Guadalajara, Jalisco a 03 de Septiembre de 2012

CONSEJO GENERAL DEL POSGRADO
INTERINSTITUCIONAL EN CIENCIA Y TECNOLOGÍA
PRESENTE

El que suscribe, Dr. Alberto López López, en calidad de director de la tesis denominada: "COMPUESTOS EMERGENTES: OPTIMIZACIÓN DE MÉTODOS ANALÍTICOS PARA LA DETERMINACIÓN EN MUESTRAS DE AGUA Y EVALUACIÓN DE CINÉTICA DE OXIDACIÓN USANDO OZONO**"** del estudiante **Ramiro Vallejo Rodríguez**, autoriza la impresión del documento una vez que ha sido revisado y corregido.

Esperando que la presente sirva a su portador, quedo al pendiente para cualquier duda o aclaración al respecto. Aprovecho la ocasión para enviar1e un cordial saludo.

_____

Dr. Alberto López López
Director de tesis

CIENCIA Y TECNOLOGIA

Guadalajara, Jalisco a 05 de Septiembre de 2012

CONSEJO GENERAL DEL POSGRADO
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PRESENTE

Los abajo firmantes miembros del Jurado del Examen de Grado del estudiante **Ramiro Vallejo Rodríguez**, una vez leída y revisada la Tesis titulada **"**COMPUESTOS EMERGENTES: OPTIMIZACIÓN DE MÉTODOS ANALÍTICOS PARA LA DETERMINACIÓN EN MUESTRAS DE AGUA Y EVALUACIÓN DE CINÉTICA DE OXIDACIÓN USANDO OZONO**",** aceptamos que la referida tesis revisada y corregida sea presentada por el alumno para aspirar al grado de Doctor en Ciencia y Tecnología en la opción terminal de Ingeniería Ambiental durante el examen correspondiente.

Y para que así conste firmamos la presente a los 05 del mes de septiembre del año 2012.

Dr. Javier José Navarro Laboulais *Presidente*	Dra. Gabriela Eleonora Moeller Chávez *Secretario*
Dr. Hugo Albeiro Saldarriaga Noreña *Vocal*	Dr. Mario Alfonso Murillo Tovar *Vocal*

Dr. Alberto López López
Vocal

A mis padres Daniel y Rosario:

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- Vallejo-Rodríguez, R y López-López, A. (2009), "Compuestos disruptores endócrinos: identificación y análisis en agua superficial y su degradación utilizando un proceso de oxidación con ozono" en Retos de la Investigación del Agua en México/ Úrsula Oswald Spring, coordinadora. Cuernavaca: UNAM, Centro Regional de Investigaciones Multidisciplinarias, 2011. 754 p. ISBN: 978-3-926979-81-0.

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- Vallejo-Rodríguez R., Alberto Lopez-Lopez A., Navarro-Laboulais J., Esplugas S., Science of the Total Environment, Assessment of the kinetics of oxidation of steroids and pharmaceutical compounds in water using ozone, In review, http://ees.elsevier.com/stoten/default.asp, Manuscript Number: STOTEN-D-12-02514.

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ABBREVIATIONS

AOPs: advanced oxidation processes

AOP-O_3: Advanced oxidation processes using ozone:

CCL: contaminant candidate list

CV: coefficient of variation

DAD: diode array detector

E2: 17β-Estradiol

ECs: emerging contaminants

EDCs: endocrine disrupting compounds

EE2: 17α-Ethinylestradiol

GC: gas chromatography

HLB: hydrophilic lipophilic balance

HPLC: high performance liquid chromatography

IBP: ibuprofen

LC: liquid chromatography

LOD: limit of detection

LOQ: limit of quantification

MS: mass spectrometry

MWTP: municipal wastewater treatment plants

n: stoichiometric coefficients

NPX: naproxen

SPE: solid phase extraction

SPME: solid phase microextraction

SDB: styrene divinylbenzene

USEPA: United States Environmental Protection Agency

WHO: World Health Organization

WWTP: wastewater treatment plants

ABREVIACIONES

CEs: compuestos emergentes:

CG: cromatografía de gases:

CL: cromatografía de líquidos:

CLAR: cromatografía de líquidos de alta resolución:

EM: espectrometría de masas

EFS: extracción en fase sólida

PAOs: procesos avanzados de oxidación

RESUMEN

Esta tesis doctoral se desarrolló en el contexto de la problemática ambiental y de salud humana relacionadas con los compuestos emergentes (CEs) presentes en el agua los cuales poseen características disruptoras potenciales. Particularmente este trabajo estableció la optimización de las condiciones analíticas para determinar esteroides y fármacos en muestras de agua usando extracción en fase sólida (EFS) y cromatografía de líquidos de alta resolución (CLAR) y la evaluación de la cinética de oxidación de estos compuestos en agua utilizando ozono.

El trabajo de investigación se divide principalmente en tres partes: una introducción general, tres capítulos generales y conclusiones generales. La contribución principal de nuestra investigación se enfoca en el establecimiento de la cinética de oxidación de los compuestos seleccionados.

En primer lugar, el capítulo uno presenta el estado de la arte sobre la aplicación de métodos analíticos para la determinación de CEs y compuestos disruptores endócrinos (CDEs) y su degradación por procesos avanzados de oxidación (PAOs). Actualmente, los CEs y CDEs representan un problema ambiental y de salud pública. Los niveles de concentraciones de dichos compuestos son muy bajos ($\mu g\ L^{-1}$-$ng\ L^{-1}$), generando complicaciones en su identificación y cuantificación. Este problema de detección ha dado lugar al desarrollo de métodos analíticos que aprovechan las ventajas ofrecidas por herramientas tales como la *cromatografía de gases* (CG) o la *cromatografía de líquidos* (CL), acoplada a *espectrometría de masas* (EM). Por su parte, para la degradación de los CEs y CDEs, los procesos de avanzados de oxidación utilizando ozono (AOP-O_3) son los más estudiados debido a su alta eficiencia para oxidar estos compuestos (> 80% en períodos de tiempo relativamente cortos del orden de minutos.

En el capítulo dos se presenta la metodología para la determinación y cuantificación de los CEs y CDEs. Se optimizaron dos métodos confiables con el fin de determinar dos esteroides (17β-estradiol y 17 α-etinilestradiol) y dos fármacos (ibuprofeno y naproxeno) utilizando EFS para la preparación de la muestra y CLAR para el análisis. Los métodos optimizados incluyeron la extracción y determinación de fenolato de sodio, que fue el reactivo utilizado en la cinética competitiva implementados en la etapa experimental

(capítulo tres). El método analítico optimizado fue aplicado a la evaluación preliminar de un ensayo de oxidación de un esteroide (17β-estradiol) utilizando ozono, encontrando que el límite de detección estimado es suficiente para determinar la masa residual ($\mu g\ L^{-1}$) del esteroide después del tratamiento.

El capítulo tres presenta el objetivo de esta investigación el cual fue la evaluación de la cinética de oxidación de cuatro CEs en agua enriquecida (dos esteroides y dos fármacos) mediante procesos de ozonización. Se obtuvieron la estequiometría y las constantes de velocidad de segundo orden de los cuatro compuestos seleccionados. Con el fin de evaluar la cinética de oxidación, la metodología de cinética competitiva se utilizó para los esteroides y naproxeno; las constantes absolutas de velocidad bajo condiciones de pseudo-primer orden se establecieron para cinética de oxidación de ibuprofeno. El modelo de oxidación obtenido de los CEs de las constantes de velocidad fue representado para dosis diferentes de ozono y pudo compararse con los valores experimentales. Además, se obtuvo la vida media de la CEs seleccionados para cada dosis de ozono aplicada.

Finalmente, se establecieron las conclusiones generales las que son satisfactorios en el cumplimiento de los objetivos iniciales: la optimización de los métodos analíticos para los CEs seleccionados y la evaluación de la cinética de oxidación de los compuestos modelo. Estas dos últimas aseveraciones son la contribución de esta investigación científica y forma parte de un proyecto orientado hacia el tratamiento de agua conteniendo CEs a escala semi-piloto en régimen continuo.

SUMMARY

This Doctoral Thesis is developed in the context of environmental and human health problems related with Emerging Compounds (ECs) in water with potential disrupting characteristics. Particularly this work established the optimization of analytical conditions to determine steroids and pharmaceuticals drugs in water samples using solid phase-extraction and high performance liquid chromatography and the assessment of the kinetics of oxidation of those compounds in water using ozone.

This Thesis is divided in three mainly parts, the general introduction, three chapters and general conclusions. The principal contribution of our research concerns the establishment of oxidation kinetics of selected compounds.

Firstly, chapter one presents the state-of-the-art about the identification of analytical methods for determining ECs and endocrine disrupting compounds (EDCs) and their degradation by *advanced oxidation processes* (AOPs). As mentioned above, today ECs and EDCs are environmental and public health problems; also, their concentrations in water are very low ($\mu g \cdot L^{-1}$-ng L^{-1}), generating complications in their identification and quantification. This detection problem has given rise to the development of analytical methods that utilize such tools as *gas chromatography* (GC) or *liquid chromatography* (LC), coupled with *mass spectrometry* (MS). For degradation of ECs and EDCs, the *advanced oxidation processes using ozone* (AOP-O_3) are the most studied because of their high efficiency to oxidize these compounds, greater than 80 per cent, in relatively short time periods, in the order of minutes.

Methodology for the determination and quantification of EDCs is presented in chapter two. Two reliable methods were optimized in order to determine two steroids (17β-Estradiol and 17α-Ethinylestradiol) and two pharmaceutical drugs (ibuprofen and naproxen) using Solid-Phase Extraction (SPE) for sample preparation and High Performance Liquid Chromatography (HPLC) for analysis. The optimized methods include the extraction and determination of sodium phenolate, which was the reagent used in competitive kinetics implemented in the experimental stage (chapter three). The optimized

analytical method was applied to the preliminary evaluation of a steroid oxidation test using ozone, finding that the estimated limit of detection is sufficient to determine the residual mass ($\mu g \cdot L^{-1}$) of 17β-Estradiol after the experiment.

Chapter three presents the aim of this research which was the assessment of the oxidation kinetics of four ECs in spiked water (two steroids and two pharmaceutical compounds) by ozonation processes. The stoichiometry and the second order rate constants of the four selected compounds were obtained. In order to assess the oxidation kinetics, competitive kinetics methodology was used for steroids and naproxen; absolute rate constant under pseudo-first order conditions were established for ibuprofen oxidation kinetics. The oxidation model obtained for the ECs from the rate constants was represented for different ozone doses and could be compared to the experimental values. In addition, the half-life of the selected ECs for each applied ozone dose was obtained.

Finally, general conclusions are established which are satisfactory in meeting the initial objectives: optimization of analytical methods for selected ECs and evaluation of the kinetics oxidation of model compounds. These last two statements are the contribution of this scientific research and takes part of a project targeted towards water treatment containing ECs at semi-pilot scale under continuous regime.

GENERAL INTRODUCTION

Occurrence on the environment of ECs and EDs

Currently, there are a lot of studies that indicate the occurrence of new contaminants or Emerging Contaminants (ECs) and Endocrine Disrupting Compounds (EDCs) in surface waters, ground waters and wastewaters. The United States Environmental Protection Agency (USEPA) has defined endocrine disruptor as an exogenous agent that interferes with the synthesis, secretion, transport, association, action, or elimination of natural hormones in the body, which are responsible to maintain homeostasis and reproduction (USEPA, 1997). The term "Emerging Contaminants" does not necessarily correspond to "new substance", i.e., newly introduced chemicals and their degradation products/metabolics or by-products, but also refers to compounds with previously unrecognized adverse effects on the ecosystems, including naturally occurring compounds. The "emerging contaminants" can be defined as contaminants that are currently not include in routine monitoring programmes and which may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects, public perception and on monitoring data regarding their occurrence in the various environmental compartments (Petrovic *et al*, 2008). Today ECs are an object of study. This group of contaminants includes natural estrogens, pharmaceuticals drugs, veterinary drugs, antiseptics, and personal care products. ECs are not necessarily endocrine disruptors, but their low biodegradability contributes to the disruption of the endocrine systems of living (Acosta *et al*., 2005; Daughton, 2005).

A large number of studies at laboratory level have also reported the effects of ECs and EDCs and on the endocrine systems of fish, reptiles, birds, and mammals (disruption of reproductive functions), such as: sexual differentiation, ovarian function, production of sperm and fertilization (Guzmán/Zambrano, 2007; Anway, 2006; Acosta *et al*., 2005). There is evidence for anomalies and disturbances in the human endocrine system related to these substances, characterized by changes in the hormonal content in the thyroid and in the male and female reproductive systems, demonstrated by high incidences of breast cancer, prostate and testicular cancer, male infertility, and reduction in the production of sperm (Guzmán/Zambrano, 2007; Acosta *et al*., 2005; Mitra *et al*., 2004).

In the last decade, the effects of ECs and EDCs on living organisms have become more evident to the United States Environmental Protection Agency (USEPA, 2008; 2007) and in particular the Office of Investigation and Development has considered this theme as one of the six scientific research priorities in the United States. For this reason, there is an increased interest, particularly for the EDCs, among the international scientific community. The Safe Drinking Water Act directs USEPA to publish a Contaminant Candidate List (CCL) every five years. The drinking water CCL is a list developed by EPA that identifies priority contaminants for regulatory decision making and information collection. They published the first CCL in March 1998, the second CCL in February 2005, and the draft CCL 3 was published in February 2008. The contaminants on the list are known or anticipated to occur in public water systems and may require regulation. For those contaminants that lack sufficient information, EPA will encourage research to provide the information needed to determine whether to regulate the contaminant (USEPA, 2009; 2008).

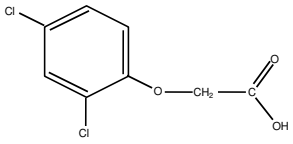
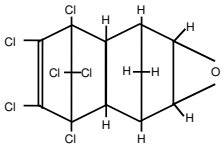
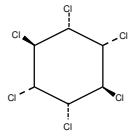
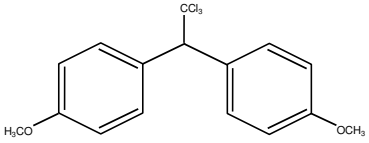
The ECs and EDCs are generated by anthropogenic activities and come from of various sources of pollution: domestic sewage and industrial effluents treated or untreated, runoff from cultivated areas, use of sludge in agricultural areas, among other sources, which eventually flow into surface waters that are used for potable water or irrigation (Benotti *et al*., 2009; Esplugas *et al*. 2007). Diverse studies have recently reported ECs and EDCs in samples of domestic wastewaters and effluents from municipal wastewater treatment plants in various regions of Europe, Asia and America (Xu *et al*., 2012; Kim *et al*., 2007; Nakada *et al*., 2006), but mostly persist and are found in surface water in concentrations of μg/L o ng/L. Furthermore, surface water are the source of potable water supplies, thereby putting the populations that consume this water at risk (Basile *et al*., 2011; Benotti *et al*., 2009; Sharma, 2008; Kim *et al*., 2007; Gibson *et al*., 2007).

The ECs includes pharmaceuticals drugs and their increase in demand worldwide also implies an increase of the wastes thereof by the population. Pharmaceuticals drugs are partially metabolized and excreted through the feces and urine in wastewater (Benotti *et al.,* 2010; Samaras *et al*., 2010). The expired drugs are dumped into the sanitary sewers and wastewaters are finally discharged into treatment plants.

In 2010, the global turnover of pharmaceutical drugs totaled 861 billion US-Dollars, an increase of 5.4 % compared to the previous year. Nearly 80 % of the total turnover of the global pharmaceutical market is generated by North America, Europe and Japan. Latin America increased its pharmaceutical turnover significantly by 21 % to almost 33 billion Euros (Pharma Data, 2011). Statistics show that drug consumption worldwide is increasing. The problem of environmental and human health, and waste treatment also increased due to raised volume of waste generated by the use and abuse of pharmaceutical drugs.

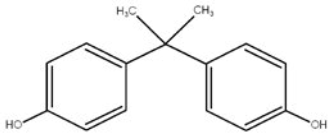
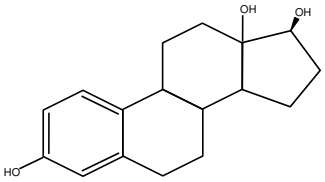
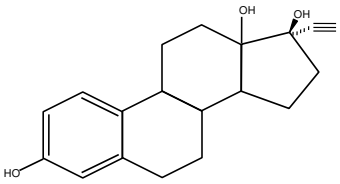
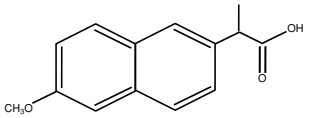
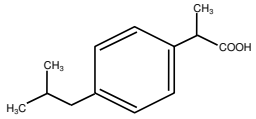
Table 1 summarizes the solubility in water and structure of some representative ECs and EDCs in environmental samples. The compounds that have been declared endocrine disruptors are pesticides 2,4-D (2,4-dichlorophenoxyacetic acid), endrin, lindane, methoxychlor (USEPA, 2000; 1997; Rawlings *et al*. 1998); and steroids 17β-estradiol and 17α–ethinylestradiol (Brion *et al*., 2004; Lange *et al*., 2000; Lindholst *et al*., 2000). The effects of disruption of antibiotics and other pharmaceuticals are currently under study (Eckstein/Sherk, 2011; Nakada *et al*., 2006; Snyder et al., 2006; 2005; Daughton, 2005; McCormick et al. 2005).

Table 1. Values of pKa, solubility in water and structure of selected compounds

Compound (use)	Solubility in water (mg L^{-1}) 25 °C	Structure
2,4-D	871[a]	
Endrin	2.60 x 10^{-3} [b]	
Lindane	10 [a]	
Metoxychlor	1.00 x 10^{-3} [b]	

[a] Linde 1994; [b]Altschuh *et al.* 1999

Table 1. Values of pKa…(continuation)

Bisphenol A	120-300 [c]	
17β-Estradiol	1.51 [d]	
17α-Ethinylestradiol	9.20 [d]	
Naproxen	15.19 [e]	
Ibuprofen	4.75 [f]	

[c]Staples *et al*., 1998; [d]Shareef *et al.* (2006); [e] Kim (2008); [f]Garzón/Martínez (2004)

Based on the foregoing, the compounds used in this work were selected according to their commercial demand, frequency of use, and presence in bodies of water. These were two steroids: one natural, 17β-Estradiol (E2), and one synthetic, 17α-Ethinylestradiol (EE2) (Benotti *et al*. 2010; Jafari *et al*. 2009); and two anti-inflammatory pharmaceuticals, naproxen and ibuprofen (Samaras *et al*. 2010; Radjenović *et al*., 2009, Santos *et al*. 2005).

Analytical methods for determining ECs and EDCs

The low concentration of ECs represents an analytical problem, besides the chemical interference of organic compounds in aqueous matrices. There are no standardized methods to analyze groups of ECs, which means that more specific analytical methods need to be implemented.

Due to the low concentrations of the EDCs and ECs in water, the extraction procedures are generally applied for concentrations of compounds of interest in an aqueous matrix. Currently, different types of analytical instrumentation can be used for measuring the compounds of interest in the extracts. However, *mass spectrometry* (MS) or MS in tandem with *gas chromatography* (GC) or *liquid chromatography* (LC) have become the most commonly used instrumentation for the analysis of these traces compounds (Yu, 2007).

In general, the identification and quantification of ECs and EDCs includes sampling, chromatographic separation, and final detection (Samaras *et al.*, 2010; Gibson *et al*., 2007; Yu, 2007). Methods like *solid phase extraction* (SPE) and *solid phase microextraction* (SPME) techniques are more employed in the extraction and concentration steps of analytes.

The SPE is a technique that allows concentrating and purifying an aqueous sample by absorption of a cartridge in solid phase, followed by elution of the analyte with a suitable solvent and subsequent instrumental analysis (Thurman/Mills, 1998). There are a variety of cartridges that allow the extraction and purification of the compounds according to the polarity and dissociation constant of the analyte, and the pH of the aqueous sample. In the analysis of EDCs and ECs, octadecyl (C18) bonded silica cartridges have been widely used for extraction from water samples (Huerta-Fontela *et al*., 2011; Kusk *et al*. 2011; Samaras *et al.* 2010; Tian *et al*. 2010).

A more recent extraction technique, solid-phase microextraction (SPME), was introduced by Pawliszyn, (1997). SPME consists of an absorption and a desorption step (Peñalver *et al*., 2002). Usually, SPME is combined with GC, placing the fiber in the hot injector of the gas chromatograph, where the analytes are thermally desorbed.

During the last decade GC and LC methods, coupled with MS, have been developed; these are viable in time and resources and avoid complicated extraction techniques, sublimation of solvents, and liquid-liquid extractions (Liu *et al*., 2009; Yu, 2007).

However, the implementation of an analytical method for determining EDCs and ECs in a laboratory is related with technical and available economic resources. The optimization of the analytical methods used in this research has considered the above

factors. The determination of model compounds was carried out by HPLC and diode array detector (DAD) considering the availability of this equipment in laboratories and chemical characteristics of the model compounds.

Ozone in wastewater treatment

It has not been simple to degrade ECs and EDCs using conventional treatment processes, such as biological and physicochemical processes, because of their low biodegradability (Benotti *et al*., 2009; Kim *et al*., 2007; Nakada *et al*., 2006). In this context, water pollution from ECs and EDCs also represents a technical problem for purification and treatment of water and wastewater, given that the conventional aerobic process, coagulation-flocculation, filtration, and disinfection with chlorine treatments are not capable of removing or degrading the compounds (Benotti *et al*., 2009; Kim *et al*., 2007). In this regard, attempts have been made in recent years through research studies to establish a water treatment process for surface water contaminated with ECs and EDCs (Maniero-Ferreira *et al*., 2009; Rivas *et al*., 2009; Sharma, 2008). Thus a*dvanced oxidation processes* (AOP) and ozonation emerged as a necessary treatment. The AOPs and ozonation used until now seem to be the most viable processes because of their high efficiency (more than 80 per cent) obtained in relatively short times (of the order of minutes) for the degradation of ECs and EDCs.

AOP processes are based on the coupling of two or more oxidizing agents (O_3/pH↑, O_3,/H_2O_2, Fe_2^+/H_2O_2, O_3/Cat, H_2O_2/UV, O_3/UV) in order to generate hydroxyl radicals ($OH^{\bullet}$), the principal species that cause rapid and complete oxidation of recalcitrant or difficult-to-biodegrade compounds, including ECs and EDCs (Maniero-Ferreira *et al*., 2009; Rivas *et al*., 2009; Sharma, 2008).

The ozone molecule represents a hybrid, formed by the four possible structures. The resonance forms of the ozone molecule confer some sort of polarity. Different properties of molecules (solubility, type of reactivity of bonds, etc) are partially due to the polarity that is measured with the dipolar momentum. The ozone molecules present a weak polarity (0.53 D), probably due to the electronegativity of oxygen atoms and the unshared pairs of electrons in some of the orbitals (Beltrán, 2004).

The O_3 molecule and the $OH^{\bullet}$ radical have the potential for oxidation-reduction (E°) of 2.07 and 2.8 volts, respectively; fluorine has a more elevated potential (3.0), and these are the three oxidant species with the highest potentials (Vanýsek, 2011).

The high reactivity of ozone can be attributed to the electronic configuration of the molecule. Thus, the absence of electrons is one the terminal oxygen atoms in some of the resonance structure confirm the electrophilic character of ozone. These properties make ozone an extremely reactive compound (Beltrán, 2004). Table 2 presents some physic-chemicals properties of ozone.

Table 2. Physico-Chemical Properties of Ozone (adapted from various sources)

Property	Value
Molecular weight, g mol^{-1}	48.0
Melting point, °C [a]	-251
Boiling point, °C [a]	-112
Critical pressure, atm [a]	54.62
Critical pressure, °C [a]	-12.1
Specific gravity [a]	1.71 g cm^{-3} a -183 °C
Oxidation potential, V [a]	2.07
Instant odor threshold [b]	40 μg m^{-3}
Permissible exposure limit (average over 8 h workshift)	0.1 ppm (200 μg m^{-3}) [c] 0.05 ppm (100 μg m^{-3}) [d]
Redox potential in aqueous reduction [b]	E_0 = 2.07 V
Explosion hazard [e]	10-11% (v/v) in oxygen (760 torr)

[a] Perry/Green, 1997; [b] Hoigné 1998; [c] OSHA, 2012; [d] WHO, 2012; [e] Koike *et al.* 2005

Ozone is a highly toxic gas; exposure to ozone can irritate eyes, noses, and throat. Breathing ozone can irritate the lungs and cause coughing and/or shortness of breath. The previously recommended limit, which was fixed at 120 μg/m^3 8-hour mean, has been reduced to 100 μg m^{-3} based on recent conclusive associations between daily mortality and ozone levels occurring at ozone concentrations below 120 μg m^{-3}(WHO, 2012). Therefore, the management of ozone for experimental purposes should be following the limits recommended by health and labor organizations.

Because of its high standard redox potential, the ozone molecule has a high capacity to react with numerous compounds. This reactivity is particularity important in the case of some inorganic compounds. In most of these reactions, there is oxygen transfer from the ozone molecule to the other compound (Beltrán, 2004).

The action of ozone on organic material in aqueous media has been thoroughly studied; it has been concluded that the oxidation reaction occurs by molecular or radical pathway. The molecular pathway (direct reaction) acts selectively on organic compounds that have double bonds and are prevalent at low pH. For the radical pathway (indirect reaction), the principal oxidizing agent is the radical $OH^{\bullet}$ that acts in a non-selective form on organic compounds (Beltrán *et al*., 2008, Beltrán, 2004; López-López *et al*, 2004, Staehelin/Hoigné, 1985).

Ozone is particularly reactive toward phenols, amines, compounds exhibiting C=C double bonds, and activated aromatic compounds (e.g., polyaromatic compounds and benzene rings substituted with an alkoxy group or several aliphatic moieties) (Huber, 2004). The reaction between ozone and any olefinic compound could be an example of a cycloaddition reaction and this reaction follows the mechanism of Criegge (Beltrán, 2004; Kuczkowski, 1984).

With many inorganic compounds ozone reacts by an apparent oxygen transfer mechanism. Reactions with organic compounds usually proceed through ozone addition followed by fast rearrangement, which can result in the release of oxygen. Ozone reacts rarely by electron transfer reactions. Exceptions are the reaction of ozone with amines and phenols (Beltrán, 2004; Muñoz/von Sonntag, 2000).

Ozonation of phenols results first in the formation of benzoquinones, hydroquinones and muconic acid derivatives (Mvula/von Sonntag, 2003). Further oxidation leads to cleavage of the cyclic products and finally yields various acids and aldehydes (e.g. formic acid, glyoxylic acid, glyoxal) (Yamamoto *et al*. 1979). The reaction of ozone with tertiary amines seems to yield aminoxides or secondary amines and the corresponding aldehydes (Muñoz/von Sonntag, 2000).

The free radical species, including radical $HO^{\bullet}$, are formed in the initiation and propagation reactions of the mechanisms of AOP involving ozone and others agent, such as hydrogen peroxide or UV radiation, among others (Beltrán, 2004). In the ozone

decomposition mechanisms, the hydroxyl radical is the main responsible specie in the radical pathway. Hydroxyl radicals react with saturated organic compounds principally by H-abstraction. In the presence of oxygen, this reaction leads to the formation of peroxyl radicals, which decay either through release of superoxide or bimolecular termination. In both cases, the main products are ketones, aldehydes and alcohols. In the case of benzene, hydroxyl addition can lead to ring cleavage or the formation of phenol (Huber *et al*. 2004).
In lab-scale experiments, ozone reactions can be distinguished from hydroxyl radical reaction using a scavenger compound, which quenches hydroxyl radical without promoting the chain reaction. In this study, terbutyl alcohol was used as radical scavenger, because the experiments performed were only tested for the molecular pathway. Others compounds should be used as scavengers as acetone or the inorganic ions bicarbonate and carbonate (Huber *et al*. 2004; Staehelin/Hoigné, 1985).

The water treatment and wastewater containing EDCs or ECs by ozonation processes requires the obtaining of the reaction kinetics of the selected compounds. The reaction rate constants may be required in subsequent tests of wastewater treatment on a pilot scale. Water treatment with ozone depends mainly of the kinetics of oxidation and the liquid-gas transfer phenomenon (Beltrán, 2004), nonetheless, this research analyzes only the kinetic study without discrediting the importance of the second term.

ECs ozonation processes kinetic

Ozone reactions in water and wastewater are heterogeneous parallel-series gas liquid reactions in which a gas component (ozone) is transferred from the gas phase (oxygen or air) to the liquid phase and simultaneously reacts with other substance (pollutants) while diffusing. The main aim of the kinetic study is to determine the constant rate of reaction and stoichiometric coefficient. This is achieved by establishing the corresponding kinetic law. In contrast to chemical equilibrium, kinetics laws are empirical and must be determined from experiments. According to the type of experiments, the ozonation kinetic study can follow one of two different approaches (Beltrán, 2004). The first approach is based on experiment results of *homogeneous* ozonation reactions. This is the case where ozone and any compound are dissolved in water and then mixed and their concentrations with time are observed. The kinetic law, in this case, relates the chemical reaction rate to

the concentration of reactants (and products, in the case of reversible reactions). Thus, for any general irreversible ozone direct reaction with a compound M,

Eq. (1)

here *n* is the stoichiometric coefficients of ozone, and M_{ox} is the products of oxidation in the reaction. The kinetic laws corresponding to the ozone or M chemical reaction rates are,

Eq. (2)

and

Eq. (3)

here *k*, *n*, and *m* are the reaction rate constants and reaction orders for ozone and M, respectively. Equation (2) and (3) are related by the stoichiometric coefficients (n) (Beltrán, 2004):

Eq. (4)

The second possibility is the study of ozonation kinetics as a *heterogeneous* process – that is, as it is in practice. In the second case, the absorption rate of ozone or ozonation rate, , represents the kinetic law of the heterogeneous process. The stoichiometric equation is now

Eq. (5)

And Eq. (1). Equation (5) represents the mass transfer of ozone from the gas to the water phase, and k_La is the volumetric mass transfer coefficient (Beltrán, 2004).

Both the *homogeneous and heterogeneous approaches* have advantages and drawbacks. For example, the homogenous approach does not have the problem of mass transfer, and rate constants can be obtained straightforward from experimental data of concentration time. Unfortunately, this approach does not allow a comparison between mass transfer and reaction rates, and it is not suitable for very fast ozone reaction unless expensive apparatus, such as the stopped flow spectrophotometer, are available. The *heterogeneous approach* presents the problem of mass transfer and it must be considered

simultaneously with the chemical reaction, any type of ozone reaction kinetics can be studied with simple experimental apparatus (Beltrán, 2004).

When a homogeneous reaction is studied, the rate law is exclusively a function of the concentration of reactants, rate constants of the reaction, and reaction orders.

The kinetics study of homogeneous ozone reaction could preferentially be carried out in three different ideal reactors (Beltrán, 2004):

- The perfectly mixed bath reactor
- The continuous, perfectly mixed reactor
- The continuous plug flow reactor

In practice, the reactions are usually carried out in small flasks that act as a perfectly mixed batch reactor. In these reactors, the concentration of any species and temperature are constants throughout the reaction volume. This hypothesis allows the material balance of any species i, present in water to be defined as follows:

— Eq. (6)

here N_i and V are the molar amount of compounds i charged and the reaction volume, respectively, and r_i is the reaction rate of the i compound. Since ozone reactions are in the liquid phase, there is no volume variation and, hence, Eq. (6) can be expressed as a function of concentration, once divided by V:

— Eq. (7)

Another simplification of the ozonation kinetics is due to the isothermal character of these reactions so that the use of the energy balance equation is not needed.

In a general case, ozonation experiments aim at studying the kinetics of direct ozone reactions are developed in the presence of scavenger of hydroxyl radicals and/or at acid pH so that the ozone decomposition reaction to yield hydroxyl radicals is inhibited. This is so because the chemicals reaction rate, r_i, presents two contributions due to the molecular pathway (direct reaction) itself and the hydroxyl radical reaction. Thus, for an ozone-reacting compound M, the chemical reaction is

Eq. (8)

here k_{HO} and C_{HO} are the rate constants of the reaction between B and the hydroxyl radical and its concentration, respectively. Addition to the reacting medium of hydroxyl radical scavengers and/or carrying out the reaction at acid pH yields negligible or not contribution of the free radical reaction (second term on the right side in Eq. 8) to the reaction rate. For this, the kinetics of M would be due exclusively to the molecular pathway with ozone.

The determination of the rate constants > 1000 M-1s-1 can be analyzed with sophisticated equipment such as stopped-flow and quenched-flow systems. However, those techniques are expensive and there are spectral interferences between the oxidant (O_3), the analyte and the sub-products of the oxidation. On the other hand, the competition kinetics model is presented as an alternative to assess the rate constants >1000 $M^{-1}s^{-1}$ and to avoid the above issues (Benítez *et al*. 2009; Deborde *et al*. 2005; Huber *et al*. 2003; Gurol/Nekoulnaini, 1984; Hoigné/Bader, 1983). The denominated determination of the absolute rate constant is used to assess the rate constants that are <1000 $M^{-1}s^{-1}$; it can be performed in two pathways: i) by measuring analyte oxidation and maintaining high and constant ozone concentration; ii) by measuring ozone consumption at different times, maintaining high analyte concentration under pseudofirst kinetic order (Huber *et al*. 2003; Hoigné/Bader, 1983). In the state-of-technique was found that several authors have focused on the degradation of estradiol, ethinylestradiol, naproxen and ibuprofen. Oxidation techniques for the first three compounds was assayed by competitive kinetics (Benítez *et al*. 2009; Deborde *et al*. 2005, Huber *et al*. 2005, Huber *et al*. 2003), while ibuprofen, with the kinetics absolute (Huber *et al*. 2003; Nanaboina *et al*. 2010).

General introduction shows a sketch of the health and environmental problems caused by the presence of ECs and EDCs in environmental water samples. These issues represent an unresolved problem of analysis and degradation of these pollutants in various kinds of waters. Therefore, there are presented below general and specific objectives that raised this scientific research.

OBJECTIVES

The main purpose of this study was to obtain the kinetics of oxidation reaction using ozone of the four selected pharmaceuticals drugs, considering that further research will use the

kinetics constants obtained here and this issue is the principal contribution of our research. The specific objectives were:

- to investigate the state-of-the-art about the analytical methods for identification and quantification of EDCs and ECs and their oxidation by POA-O_3;
- to optimize of analytical conditions to quantification of steroids and pharmaceuticals drugs, and oxidizing species used in the experimental phase;
- to assess the stoichiometry and second order rate constants of oxidation of the four selected compounds in spiked solutions using ozone;
- to establish a mathematical model of the four model compound to represent the oxidation kinetics using ozone.

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CHAPTER 1

ENDOCRINE DISRUPTING COMPOUNDS: IDENTIFICATION AND ANALYSIS IN SURFACE WATERS AND THEIR DEGRADATION BY ADVANCED OXIDATION PROCESS WITH OZONE

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Abstract

This chapter presents the state-of- the-art techniques for identification and analysis of *endocrine disrupting compounds* (EDCs) and their degradation by *advanced oxidation processes* (AOPs). Today EDCs are an environmental and public health problem. Their concentrations in water are very low (μg/L-ng/L), which complicates their identification and quantification. This analytical problem has given rise to the development of analytical methods that utilize such tools as *gas chromatography* (GC) or *liquid chromatography* (LC), coupled with *mass spectrometry* (MS). The most commonly used are GC/MS, GC/MS/MS in sequence or LC-MS/MS. The use of the solid-phase extraction technique reduces the amount of time and the use of resources compared conventional methods. The *advanced oxidation processes that use ozone* (AOP-O_3) are the most studied because of their high efficiency, greater than 80 per cent, in relatively short time periods - off on the order of minutes. AOP-O_3 is capable of achieving partial or total (mineralization) oxidation of the organic material. AOP-O_3 promise to be one of the most appropriate technological resources not only for treating surface waters containing EDCs, including hormones and pharmaceuticals, but also for industrial effluents in general, contaminated with compounds of low biodegradability.

Introduction

The United States Environmental Protection Agency (USEPA) has defined *endocrine disrupting chemicals* (EDCs) as exogenous agents that interfere with the synthesis, secretion, transport, binding, action or elimination of natural hormones responsible for maintaining homeostasis and reproduction in live beings (USEPA, 1997).

Anthropogenic activities, particularly industrial activities, generate and utilize a large range of EDCs, such as: alkylphenols, dioxins, bisphenol A, *polycyclic aromatic hydrocarbons* (PAHs), styrene and phthalates. Numerous investigations have reported the effects of EDCs on the endocrine system of human beings such as: sexual differentiation, ovarian function, sperm production and fertilization, cryptorchidism, hypospadia, including changes in thyroid hormones (Morales-Suárez-Varela *et al*. 2011; Bourguignon/Parent 2010; Guzmán/Zambrano, 2007; Anway, 2006; Acosta *et al*., 2005; Daughton, 2005; Mitra *et al*., 2004; Ibarluzea *et al*., 2004; Sweeney, 2002; Harrison *et al*., 1997).

'New' contaminants or 'emerging contaminants' (ECs) also exist which represent a risk to humans and are not regulated by competent sanitary and environmental authorities. Today ECs are an object of study (USEPA 2009, 2008). This group of contaminants includes natural estrogens such as 17β–estradiol, estrona, estriol; synthetic estrogens such as 17α-ethinylestradiol and mestranol, in addition to some pharmaceuticals, veterinary

drugs, antiseptics and personal care products. ECs are not necessarily endocrine disruptors but their low biodegradability contributes to the presence of the disruption of the endocrine systems of living organisms, as in the case of carbamazepine, diclofenac, diazepam, carbamazepine, gemfibrozil, to name a few (Eckstein/Sherk, 2011; USGS, 2008; Esplugas *et al*., 2007; Daughton, 2005; Petrovic *et al*., 2004; Ibarluzea *et al*., 2004; Schlumpf *et al*., 2004; Campbell III/Kraus, 2002; Johnston *et al.* 2002).

EDCs and ECs are dumped directly into the environment by various anthropogenic activities, and even though some industrial and domestic effluents are previously treated, these compounds persist and are found in effluents from *wastewater treatment plants* (WWTP) (Al-Odaini *et al*., 2011; Benotti *et al*., 2009; Gatidou *et al*., 2007; Miao *et al.* 2005; Alda/Barcelo *et al*. 2001). As a consequence, these compounds have been identified in surface water (lakes, rivers, lagoon systems, etc.) (Moring, 2012; Matamoros *et al*., 2010; Benotti *et al*., 2009; Suárez *et al*., 2007; Bila *et al*., 2005; Kashiwada *et al*., 2002; Williams *et al*., 1999); by leaching, these compounds reach groundwater (Hsing-Chang *et al*., 2009; Gibson *et al.* 2007; Sacher *et al.* 2001).

One problem in the detection techniques and analysis of EDCs in water is their low concentrations (μg L^{-1} and ng L^{-1}l), in addition to the interferences by other organic compounds, which means that more specific analytical methods need to be implemented. During the last decade *gas chromatography* (GC) and *liquid chromatography* (LC) methods, coupled with *mass spectrometry* (MS), have been developed; these are viable in time and resources avoiding complicated extraction techniques, sublimation of solvents and liquid-liquid extractions (Al-Odaini *et al*. 2011, Daneshvar *et al*. 2010, Kusk *et al.* 2011, Liu/Mizutani, 2009; Gibson *et al*., 2007; Yu, 2007).

It has not been simple to degrade EDCs and ECs using conventional treatment processes, such as biological and physiochemical, because of their low biodegradability (Basile *et al.* 2011; Benotti *et al.*, 2009; Kim *et al.*, 2007; Nakada *et al*., 2006). Thus, AOPs arose as a necessary treatment. Studies are now available of the implementation of various AOPs at laboratory level for degrading EDCs and recalcitrant organic matter, or material of low biodegradability in general. The AOPs used until now seem to be the most viable because to the high efficiency (more than 80 per cent) obtained in relatively short

times (of the order of minutes) for the degradation of EDCs and ECs (Kim *et al.* 2011; Maniero *et al*., 2009; Bila *et al*., 2005; Kim *et al*. 2004; Huber *et al*., 2003).

This chapter presents the state-of-the-art techniques for identification and analysis of endocrine-disrupting compounds present in surface water and their degradation by means of advanced oxidation processes using ozone.

1. Problems

1.1. Environmental and Public Health

A large number of studies on a laboratory level have also reported the effects of EDCs and ECs on the endocrine systems of fish, reptiles, birds, and mammals (disruption of reproductive functions) such as: sexual differentiation, ovarian function, production of sperm and fertilization (Waye/Trudeau, 2011, Caliman/Gavrilescu, 2009: Guzmán/Zambrano, 2007; Anway, 2006; Acosta *et al*., 2005; Lintelmann *et al*., 2003; Lister/Krack, 2001; USEPA, 1997).

There is evidence for anomalies and disturbances in the human endocrine system related to these substances, characterized by changes in the hormonal content in the thyroid and in the male and female reproductive systems, demonstrated by high incidences of breast cancer, prostate and testicular cancer, male infertility, and reduction in the production of sperm (Waye/Trudeau, 2011, Guzmán/Zambrano, 2007, Acosta *et al*., 2005; Mitra *et al*., 2004; Ibarluzea *et al*., 2004; Rodger *et al*., 2000; Harrison *et al*., 1997).

In the last decade, the effects of EDCs on living organisms have become more evident to the USEPA (2008, 2007) and in particular the Office of Investigation and Development has considered this theme as one of the six scientific research priorities in the United States. For this reason, there is increased interest, particularly for the EDCs, among the international scientific community. The Safe Drinking Water Act directs USEPA to publish a Contaminant Candidate List (CCL) every five years. The drinking water CCL is a list developed by EPA that identifies priority contaminants for regulatory decision making and information collection. They published the first CCL in March 1998, the second CCL in February 2005, and the draft CCL 3 was published in February 2008. The contaminants on the list are known or anticipated to occur in public water systems and may require regulation. EPA will evaluate all the contaminants on the CCL 3 to determine which

contaminants have sufficient information to allow the Agency to make a regulatory determination. For those contaminants that lack sufficient information, EPA will encourage research to provide the information needed to determine whether to regulate the contaminant (USEPA 2009, 2008).

1.2. Water treatment

A common characteristic of the EDCs is the recalcitrance or persistence against natural or controlled biological degradation (Xu *et al*. 2012; Mao *et al*. 2010; Koh *et al*. 2009; Liu/Mizutani, 2009; Esplugas *et al*., 2007; Beltrán *et al*., 2008; Snyder *et al*.; 2006). In this context, water pollution from EDCs also represents a technical problem for treatment and purification of water, given that the conventional biological aerobic, anaerobic, coagulation-flocculation, filtration and disinfection with chlorine treatments are not capable of removing or degrading the compounds (Basile *et al.* 2011; Benotti *et al.*, 2009; Kim *et al.*, 2007; Nakada *et al*., 2006; Snyder *et al*., 2005). In this regard, attempts have been made in recent years through research studies to establish a water treatment process for surface waters contaminated with EDCs (Huerta-Fontela *et al*. 2012, Rivas *et al.*, 2009; Sharma, 2008; Maniero *et al.*, 2009; Esplugas *et al.*, 2007; Coelho *et al.*, 2007; Ikehata *et al.*, 2007; Naghashkar/El-Din, 2005a; 2005b). Despite initial technical advances in this area, challenges still remain and alternatives need to be developed as part of the process of establishing a treatment that guarantees the elimination of EDCs in water so as to reduce the potential risk to public health.

EDCs and ECs are commonly found in domestic wastewaters and in effluents from *municipal wastewater treatment plants* (MWTP) (Xu *et al*. 2012; Kim *et al*., 2007; Nakada *et al.*, 2006), but mostly persist and are found in surface water in concentrations of $\mu g\ L^{-1}$ o $ng\ L^{-1}$. Furthermore, surface water are the source of potable water supplies, thereby putting the populations that consume this water at risk (Basile *et al*. 2011; Benotti *et al.*, 2009; Sharma, 2008; Kim *et al*., 2007; Gibson *et al.*, 2007). Table 1 gives a summary of the presence of EDCs and ECs in water and soil in different parts of the world, including some of the principal water sources in Mexico. The principal sources of this information are technical reports written by the U.S. Geological Survey (USGS 2002, 2008) and Petrovic *et al*. (2004).

Of the compounds presented in Table 1, the following have been declared endocrine disruptors: pesticides: 2,4-D (2,4-dichlorophenoxyacetic acid), endrin, lindane, methoxychlor (USEPA, 2000; 1997); steroids: 17β-estradiol and 17α–ethinylestradiol (Brion *et al*., 2004; Lange *et al*., 2000). The effects of disruption of antibiotics and other pharmaceuticals are currently under study (Eckstein/Sherk, 2011; Nakada *et al*., 2006; Snyder et al., 2006; 2005; Daughton, 2005; Petrovic *et al*., 2004; Ibarluzea et al., 2004).

Table 1. Occurrence of EDCs and ECs in surfaces water in different parts of the world. Source: Authors' research.

Compound	Concentration (μg/L)	Location	Reference
		Pesticides	
2,4 – D (2,4-Dichloro-phenoxyacetic acid)	3-50	Chapala Lake, México	Alvarez (2007)
	2-50	Sayula Lake, México	
	6.1	Tula City, Hidalgo, México	Gibson *et al*. (2007)
	0.042	Clackamas River, Oregon, USA	Carpenter *et al*. (2008)
	0.012	Ebro and Llobregat Rivers, Barcelona, Spain	Matamoros *et al.* (2010)
	<0.04	Llobregat River, Barcelona, Spain	Ricart *et al* .(2010)
	1.0-0.25	Devils River, Texas USA	Moring (2012)
Dieldrin	<0.080	USA	Barnes *et al*. (2002)
	<0.024	Clackamas River, Oregon, USA	Carpenter *et al*. (2008)
	0.007	Ebro and Llobregat Rivers, Barcelona, Spain.	Matamoros et al. (2010)
	<0.009	Devils River, Texas USA	Moring (2012)
Endrin	nr	Chapala Lake, México	Alvarez (2007)
	$19x10^{-6}$	South Fork Shenandoah River, Virginia, USA	Alvarez *et al*. (2008)
Lindane	$240x10^{-6}$	South Fork Shenandoah River, Virginia, USA	Barnes *et al*. (2002)
	<0.050	USA	Alvarez *et al*. (2008)
	<0.004	Devils River, Texas USA	Moring (2012)
Malathion	0.027	Mobile River, USA	McPherson *et al*. (2003)
	<0.048	Clackamas River, Oregon, USA	Carpenter *et al*. (2008)
	<0.027	Devils River, Texas USA	Moring (2012)
Methyl parathion	<0.0060	USA	Barnes *et al*. (2002)
	<0.015	Devils River, Texas USA	Moring 2012
Methoxychlor	nr	Chapala Lake, México	Alvarez (2007)
	$47 x10^{-6}$	South Fork Shenandoah River, Virginia, USA	Alvarez *et al*. (2008)

Table 1. Occurrence… (Continuation)

Compound	Concentration (μg/L)	Location	Reference
		Various Compounds	
4-Methylphenol	<0.060	USA	Barnes *et al*. (2002)
4-Nonylphenol	0.11-0.64	USA	Naylor *et al*. (1992)
	<0.01-0.92	Greats Lakes, USA and Canada	Bennie *et al*. (1997) Bennett/Metcalfe (1998), Bolz *et al*. (2001)
	<0.01-0.49	Baden- Württemberg, Germany	Barnes *et al*. (2002)
	<0.5	USA	Petrovic *et al*. (2002)
	<0.1-0.15	Catalonia, Spain	Tsuda *et al*. (2002)
	0.02-0.3	Japan	Gatidou *et al.* (2007)
	0.07	Lesvos Island, Greece	
4- Nonylphenol	<1.0	USA	Barnes *et al*. (2002)
monoethoxylate	5.22	Lesvos Island, Greece	Gatidou *et al.* (2007)
4-Nonylphenol	<1.1	USA	Barnes *et al*. (2002)
diethoxylate	3.43	Lesvos Island, Greece	Gatidou *et al*. (2007)
BPA (Bisphenol A)	0.22	Lesvos Island, Greece	Gatidou *et al.* (2007)
Nonylphenol ethoxylate	<0.06-0.60	USA	Naylor *et al*. (1992)
	<0.02-7.8	Great Lakes, USA and Canada	Bennie *et al*. (1997) Bennett/Metcalfe (1998)
	<0.1-31	Catalonia, Spain	Petrovic *et al*. (2002)
	<0.04-0.42	Japan	Tsuda *et al.* (2002)
Octylphenol	<0.005-0.084	Greats Lakes, USA and Canada	Bennie *et al*. (1997) Bennett/Metcalfe (1998)
	<0.01-0.19	Baden- Württemberg, Germany	Bolz *et al*. (2001)
	<0.02-0-09	Japan	Tsuda *et al*. (2002)
Phenol	<0.25	USA	Barnes *et al*. (2002)
		Antibiotics	
Sulfamethizole	<0.10	USA	Barnes *et al*. (2002)
	0.74	Catalonia Rivers, Spain	García-Galán *et al.* (2010)
Sulfamethoxazole	<0.10	USA	Barnes *et al.* (2002)
	$3.7x10^{-3}$	Maury River, Virginia, USA	Alvarez *et al*. (2008)
	0.168	Catalonia Rivers, Spain	García-Galán *et al.* (2010)
Sulfathiazole	<0.10	USA	Barnes *et al*. (2002)
	0.07	Catalonia Rivers, Spain	García-Galán *et al.* (2010)
Tetracycline	<0.05	USA	Barnes *et al*. (2002)
	9.89	Andalucia, Spain	Suárez *et al*. (2007)
Triclosan	0.23	USA	Barnes *et al*. (2002)
	1.12	Lesvos Island, Greece	Gatidou *et al*. (2007)
	0.003	USA	Benotti *et al*. (2009)
Trimethoprim	<0.014	USA	Barnes *et al*. (2002)
	$65x10^{-3}$	South Fork Shenandoah River, Virginia, USA	Alvarez *et al*. (2008)
	0.011	USA	Benotti *et al*. (2009)

Table 1. Occurrence… (Continuation)

Compound	Concentration (μg/L)	Location	Reference
		Pharmaceuticals	
Acetaminophen	0.016	USA	Barnes *et al*. (2002)
	<0.036	Platte River, Nebraska, USA	Vogel *et al*. (2005)
Acetilsalicilic acid	1	Germany	Ternes (1998)
Diclofenac	1	Germany	Ternes (1998)
	$4.3x10^{-4}$	USA	Benotti *et al*. (2009)
	0.046	Ebro and Llobregat Rivers, Barcelona, Spain.	Matamoros *et al*. (2010)
Gemfibrozil	<0.015	USA	Barnes *et al*. (2002)
	0.002	USA	Benotti *et al.* (2009)
Ibuprofen	1	Germany	Ternes (1998)
	<0.018	USA	Barnes *et al*. (2002)
	$0.8\text{-}2.2x10^{-3}$	Tula City, Hidalgo, México	Gibson *et al*. (2007)
	0.041	Ebro and Llobregat Rivers, Barcelona, Spain.	Matamoros *et al.* (2010)
Naproxen	0.05-0.4	Germany	Ternes *et al*. (1998)
	$0.8\text{-}0.9x10^{-3}$	Tula, Hidalgo, México	Gibson *et al*. (2007)
	0.032	USA	Benotti *et al*. (2009)
	0.097	Ebro and Llobregat Rivers, Barcelona, Spain	Matamoros *et al*. (2010)
Ranitidine	<0.01	USA	Barnes *et al*. (2002)
	<0.013	River Platte, Nebraska, USA	Vogel *et al*. (2005)
Salicylic acid	$7.8\text{-}9.6x10^{-3}$	Tula City, Hidalgo, México	Gibson *et al*. (2007)
	0.237	Ebro and Llobregat Rivers, Barcelona, Spain	Matamoros *et al.* (2010)
		Steroids	
17α-Ethinylestradiol	$0.06x10^{-3}$	Tula, Hidalgo, México	Gibson *et al*. (2007)
	$8.1x10^{-3}$	South Fork Shenandoah River, Virginia, USA	Alvarez *et al*. (2008)
	0.001	USA	Benotti *et al.* (2009)
	0.002	Llobregat River, Barcelona, Spain	Huerta-Fontela *et al*. (2011)
17β-Estradiol	<0.5	USA	Barnes *et al*. (2002)
	$0.01\text{-}0.02x10^{-3}$	Tula, Hidalgo, México	Gibson *et al*. (2007)
	$2.3x10^{-3}$	Shenandoah River, Virginia, USA	Alvarez *et al*. (2008)
	0.017	USA	Benotti *et al.* (2009)
Estriol	$3.4x10^{-3}$	River Fork Shenandoah Sur, Virginia, USA	Alvarez *et al*. (2008)
	0.026	Llobregat River, Barcelona, Spain	Huerta-Fontela *et al*. (2011)
Estrone	$0.16\text{-}0.17x10^{-3}$	Tula, Hidalgo, México	Gibson *et al*. (2007)
	$1.6x10^{-3}$	Rio Fork Shenandoah Sur, Virginia, USA	Alvarez *et al*. (2008)
	$9x10^{-4}$	USA	Benotti *et al.* (2009)
	0.046	Ebro and Llobregat Rivers, Barcelona, Spain	Matamoros *et al.* (2010)
	$3x10^{-4}$	Llobregat River, Barcelona, Spain	Huerta-Fontela *et al*. (2011)

nr: not reported
WWTP: wastewater treatment plant

2. Identification analysis of EDCs and ECs present in surface water

In general, the identification and quantification of EDCs and ECs includes sampling, chromatographic separation, and final detection (Samaras *et al.*, 2011; Gibson *et al*., 2007; Yu, 2007; Kolpin *et al*., 2002).

The techniques utilized are of different levels of complexity, sensitivity, reliability, and cost. Due to the low concentrations of the majority of the EDCs found in water, the extraction procedures generally applied are for concentrations of compounds of interest in an aqueous matrix. Different types of analytical instrumentation can be used for measuring the compounds of interest in the extracts. However, MS or MS in tandem with GC or LC have become the most commonly used instrumentation for the analysis of these traces compounds (Yu, 2007).

The processes of extraction, sublimation of the solvent, steam distillation and liquid-liquid extraction methods have been replaced by other more efficient and versatile methods like *solid phase extraction* (SPE) and *solid phase microextraction* (SPME) techniques. SPE frequently uses discs and disposable cartridges. In the analysis of EDCs, octadecyl (C18) bonded silica cartridges have been widely used for extraction (Huerta-Fontela *et al*., 2011; Kusk *et al*. 2011; Samaras *et al.* 2010; Tian *et al*. 2010; Gatidou *et al*. 2007; Mouatassim-Souali *et al*., 2003; Jeannot *et al*., 2002; Kelly, 2000).

The EFS is a technique that allows concentrating and purifying an aqueous sample by absorption of a cartridge in solid phase, followed by elution of the analyte with a suitable solvent and subsequent instrumental analysis (Thurman/Mills, 1998). There are a variety of cartridges that allow the extraction and purification of the compounds according to the polarity and dissociation constant of the analyte, and the pH of the aqueous sample.

Typically the sorbents for SPE consist of 40 μm silica gel with approximately 60 Å pore diameter. Chemically bonded to the silica gel are the phases for each mode of action. For reversed-phase sorbent, an octadecyl (C18), octyl (C8), ethyl (C2), ciclohexyl, and phenyl functional groups are bonded to the silica. Typical loading of reversed-phase sorbent varies from approximately 5% for the C2 phase to as much as 17% for the C-18 phase. The percent loading is the amount of C-2 or C-18 phase present by weight of carbon. The capacity of the sorbent in milligrams per gram (mg g^{-1}) that may be sorbed is related to both the chemistry of the phase and the loading weight of the carbon (Thurman/Mills,

1998). The widespread use of the C18 for reversed-phase sorption is a result of the popular use of C18 columns in high pressure liquid chromatographic (HPLC). For this reason, it gained immediate popularity for SPE applications (Huerta-Fontela *et al*., 2011; Kusk *et al*. 2011; Samaras *et al.* 2010; Tian *et al*. 2010; Gatidou *et al*. 2007; Koutsuba *et al*. 2003; Jeannot *et al*., 2002; Alda/Barcelo 2001; Sacher *et al*. 2001; Bolz *et al*. 2000; Kelly, 2000; Thurman/Mills, 1998). The C18 sorbents are available in both trifunctional and monofunctional form. If the pH is adjusted to acid values, it is critical to use the trifunctional silica in order to prevent hydrolysis of the hydrocarbon group from the surfaces of the sorbent. However, if polar molecules are to be sorbed and they contain basic sites, the monofunctional sorbents will have greater capacity because of the free silanol sites that are available for secondary interactions. For these reasons, the C-18 sorbents vary from manufacturer to manufacturer. Thus, the sources of the C-18 sorbent may be selected to enhance the isolation that is required (Koh *et al*. 2007; Thurman/Mills, 1998).Polymeric sorbents, such as styrene divinylbenzene (SDB) and carbon, also are used for reversed-phase SPE. These sorbent were some of the classical reversed-phase sorbent introduced in the 1960s (Boyd *et al*., 2003; Belfroid *et al*., 1999; Thurman/Mills, 1998).

For normal-phase SPE, cyanopropyl (CN), aminopropyl (NH_2), and diol functional groups are chemically bonded to the silica gel. The loading on the cyano, amino, and diol columns are sufficiently large (~6-10% as carbon) that they may sometimes be used for reversed-phase applications, especially for the removal of hydrophobic solutes from water or other polar solvents. These hydrophobic solutes would otherwise sorb too strongly to a more hydrophobic C-8 or C-18 sorbent and would be difficult to elute. Straight silica gel also is used for normal phase SPE along with Florisil (magnesium silicate), and alumina (aluminum oxide in neutral, basic, and acid forms) (Thurman/Mills, 1998).

Ion-exchange sorbents usually contain both weak and strong cation and anion functional groups bonded to the silica gel. Strong cation-exchange sorbents contain ion-exchange sites consisting of sulfonic acid groups, and weak cation-exchange sorbents contain sites consisting of carboxylic acids groups. Strong anion-exchange sites are quaternary amines, and weak anion-exchange sites are primary, secondary, and ternary amines. Strong and weak refers to the fact that strong sites are always present as ion-exchange sites at any pH, while weak sites are only ion-exchanges sites at pH values

greater or less than the pK_a, which determines whether a site contains a proton or not. The typical ion-exchange sorbent consists of a resin of a cross linked styrene-divinylbenzene polymer. Recently, several manufacturers are producing styrene-divinylbenzene-based ion-exchange SPE sorbent that are more rugged than silica-bonded sorbent because the styrene-divinylbenzene sorbents are stable from pH 1 to 13.

Actually, the hydrophilic lipophilic balance (HLB) cartridges are often used because of its wide applicability under different conditions of pH and polarity of the analytes to be extracted. Due to its advantages have been used by various researchers in the determination of drugs, steroids and various compounds in surface waters, WWTP effluent or spiked waters, either by GC/MS (Lin *et al*., 2005, Rodríguez *et al.* 2003, Jeannot *et al.* 2002; Öllers *et al*. 2001) or tandem (Trenholm *et al*. 2006, Hoai *et al*., 2003; Quintana *et al.* 2004). Application of HLB cartridge in the SPE was used in the determination of various compounds by HPLC with UV detection (Santos *et al*. 2005), liquid chromatography tandem MS (Benotti *et al*., 2009; Hsin-Chang *et al*. 2009; Miège *et al*., 2009, Trenholm *et al*. 2006, Miao *et al*., 2002) or by ultra-performance liquid chromatography (UPLC). MCX cartridges are a variant of the HLB, as it has a strong sulfonic acid group bonded to the poly(divinylbenzene-co-N-polyvinylpyrrolidone) copolymer. These were used in the determination of pharmaceuticals drugs in water and surface water sample by LC-GM-GM quantification (Al-Odaini *et al*. 2011; Daneshvar *et al*. 2010).

A more recent extraction technique, solid-phase microextraction (SPME), was introduced by Pawliszyn, (1997). SPME consists of an absorption and a desorption step. In the absorption step, a coated fused-silica fiber extracts the analytes from the sample matrix. In the desorption step, the analytes are desorbed from the fiber and introduced into the analytical column for separation (Peñalver *et al*. 2002). Usually, SPME is combined with GC, placing the fiber in the hot injector of the gas chromatograph, where the analytes are thermally desorbed.

The SPME fiber utilization in determining nonylphenols, steroids and bisphenol A with the detection by GC/MS was reported by Feitosa de Lima-Gomes *et al*., (2011), Braun *et al*., (2003), Helaleh *et al*., (2001) and Diaz/Ventura (2002a, b).

The technique LC-MS/MS has shown to be a versatile technique that is applicable for the majority of polar or thermolabile EDCs (e.g. antibiotics). However, the LC-MS/MS

method is relatively expensive. In comparison with LC, the analysis of EDCs by GC/MS, although more limited in scope, offers a useful and sensitive method for the determination of EDCs and is more affordable for the majority of laboratories. However, often the samples require additional derivatization steps following extraction to obtain the less polar and/or more volatile target compounds. The general steps in the analysis of EDCs by GC/MS are: collecting the sample, extraction, derivatization, and finally, the identification and quantification of the compounds (Liu/Mizutani, 2009; Flemming/Bent, 2003; Kolpin, *et al*., 2002).

Table 2 present a compilation of the analysis methods used by various authors for the identification of EDCs and ECs.

3. Advanced Oxidation Processes for degrading EDCs.

In response to limited availability of technology for treating surface water contaminated with EDCs in the purification process, current research is aimed at developing treatment processes to degrade these types of contaminants and reach high efficiencies in their degradation. Some of these processes are known as AOP, with its originality based on the coupling of two or more oxidizing agents (O_3/pH↑, O_3,/H_2O_2, Fe^{2+}/H_2O_2, O_3/Cat, H_2O_2/UV, O_3/UV) in order to generate hydroxyl radicals (HO•), the principle species that cause rapid and complete oxidation of recalcitrant or difficult-to-biodegrade compounds, including EDCs (Beltrán *et al*. 2012; Kim *et al*. 2011, Rivas *et al.,*2009; Sharma, 2008; Maniero *et al*., 2009; Esplugas *et al*., 2007; Coelho *et al*., 2007; Ikehata *et al*., 2007; Naghashkar/El-Din, 2005a; 2005b; López-López *et al*., 2007 and 2004; Beltrán, 2004). In particular, this section presents the state of research concerning the advanced oxidation processes based on ozone for degrading EDCs present in water, in the process of developing and validating degradation kinetic of EDCs. Below is a brief description of the scientific fundamentals of POA-O_3, not including the processes that utilize ultraviolet radiation.

Table 2. Methods of analysis used by various authors for the identification of EDCs and ECs (Source: Adapted by Yu, 2007)

Compound	Sample preparation (extraction, elution, sample volume)	Derivatization	Internal standard/ surrogate standard	LOD and LOQ (ng/L), matrix	Detection	Reference
			Steroids			
E1, E2, EE2	SDB-XC disc; methanol; 1l sample	Dimethyldichlorosi-lane (SIL A) in toluene	PCB 103	LOD: 0.2-0.6 in surface water	GC/MS-MS	Belfroid *et al*., 1999
E1, E2, EE2	C_{18} disc; methanol-water; 2.5 l sample	MTBSTFA containing 1% TBDMCS in acetonitrile	[$^2H_4$]oestrone, [$^2H_4$] 17β- oestradiol and [$^2H_4$]17α-ethinyloestradiol	N/A	GC/MS and GC/MS-MS	Kelly, 2000
E1, E2, E3, EE2	Silica cartridge C_{18}; acetonitrile in water; 200 mL sample	N/A	N/A	LOD: 10-15, in wastewater	LC-UV	Alda/Barcelo, 2001
E1, E2, EE2, NP, 4-OP	LiChrolut EN; acetone-methanol; 1- 2 L sample	Pentafluorobenzoyl chloride (PFBCl)	1,4-Bispentafluorobenzol benzene (BPFBB) as internal standard	LOD: 0.05-0.1 in surface water	HRGC/NCI-MS and GC-ECD	Kuch/Ballschmiter, 2001
E1, E2, E3, EE2 and their conjugates	Speeddisk-C18; water/acetone (4:1) and acetone; 2 l sample	50 µL Pentafluoropropio-nic acid anhydride (PFPA)	N/A	LOQ: 0.04-0.32 , in wastewater	GC/MS	Mouatassim-Souali *et al*., 2003
E1, E2, E3, EE2, and mestranol	Oasis HLB-cartridges; ethyl acetate, 2 L sample	MTBSTFA, MSTFA and others	17β-estradiol-d_4	LOQ: 2-6 ng/L in surface water	GC/MS or GC/MS-MS	Quintana *et al*., 2004
E2, E3, E2 and EE2	Bakerbond Polar Plus C18 Speedisks, 3 x 5-mL 50% methanol/50% Dichloromethane, 1 L raw water simple	N/A	3,4-$^{13}C_2$-17β-estradiol, 2,4,16,16-$^2D_4$-estrone, 2,4,17-$^2D_3$-estriol, and 2,4,16,16-$^2D_4$-17α-ethinylestradiol as internal standard	LOD: 0.47-1.27; LOQ: 1.57-4.30, in spiked samples of raw water	HPLC-MS-MS	Chia-Yang *et al*. 2007
E1, E2, E3 and EE2 and conjugated E1-3S	tC18 cartridges, 10 mL methanol and 10 ml dichloromethane, 1 L wastewater sample	N/A	Deuterated ($d_{3/4/5}$) labelled internal standards: E1-d_4, E2-d_5, E3-d_3, EE2-d_4 and E1-3S-d_4	LOD: 0.1-0.2	LC/ESI(−) /MS/MS	Koh *et al*. 2007
E3, E2, EE2, E1, DES	HLB Oasis cartridge, MTBE 60 mg, wastewater 50 mL sample	N/A	17β-Estradiol- acetate as internal standard	LOD (ESI):4-15; (APPI): 6-16 LOQ (ESI):10-60; (APPI):20-60; surfaces water and groundwater	LC-MS-MS with ESI and APPI	Hsing-Chang *et al*., 2009

Table 2. Methods of analysis… (Continuation)

Compound	Sample preparation (extraction, elution, sample volume)	Derivatization	Internal standard/ surrogate standard	LOD and LOQ (ng/L), matrix	Detection	Reference
E1, E2, EE2 and E3 and their conjugated forms	Oasis HLB cartridges 4 mL ethyl acetate / methanol (70/30 - v/v), 100 mL(influents) and 250 mL (river waters and effluents)	N/A	β-estradiol acetate, used as internal standard, perdeuterated hormones as surrogates	LOQ: in river water, 0.4-1.2; influent WWTP, 0.8-3.0; effluent WWTP 0.4-1.2.	LC-MS/MS	Miège et al. 2009
Fifty-five pharmaceuticals, hormones and metabolites	Oasis HLB (200 mg), 2x3 mL methanol, 1 L sample	N/A	Deuterated alzaprolam-d_5, chlorpromazine-d_3, diazepam-d5, carbamazepine-d_2, furosemide-d_5 atenolol-d_7 and others, as internal standard	LOQ: 0.02-20 in wastewater samples	ultra-performance liquid chromatography (UPLC)-ion trap mass spectrometer	Huerta-Fontela *et al*. 2011
Ibuprofen, phenoprofen, naproxen, ketoprofen, flurbiprofen, fluoxetine, E1 and E2	PDMS/divinylbenzene (PDMS-DVB) SPME fiber; 1 L wastewater sample	Ethyl chloroformate (ECF) as derivatizing reagent	Deuterated ibuprofen d-3 (IBUd3) as surrogate or internal standard	LOD: 95.2-154	GC-MS	Feitosa de Lima-Gomes *et al.*, 2011
Various compounds						
4-t-OP, 4-NP, BPA	C18 and polyestyrene copolymer ENV+; acetone, metabol, deionized water; 1L sample	Phenyltrimethylam monium hidroxide	Biphenyl solution	LOQ: 0.02-0.05 demineralized water	GC-MS	Bolz *et al*., 2000
4-n-NP, 4-n-OP, BPA and others	SPME	BSTFA	N/A	LOD: 10-100 Milli-Q water	GC-MS	Helaleh *et al*., 2001
Diverse compounds						
NP, NP1EO, NP2EO, NP1EC, NP2EC	SPME: PDMS 100 and 7 μm; polyacrylate (PA) 85 μm; CW-DVB 65 μm and 70 μm; PDMS-DVB, 65 μm; and DVB-CAR-PDMS 50/30 μm)	Dimethyl sulphate (DMS). diethyl sulfate (DES), and 1-methyl-3-nitro-1-nitrosoguanidine (MNNG)	4*n*-nonylphenol, 4n-nonyloxylbenzoic acid 4*n*-nonylphenolmonoethoxylate as internal standard; 4-bromophenylacetic acid as surrogate.	LOD: 20-1500 Milli-Q water	GC-MS	Díaz/Ventura, 2002a
NP, NP1EO, NP2EO, BrNP, BrNP1EO and BrNP2EO	SPME: polyacrylate (PA) 85 μm; CW–DVB, 65 μm; and (DVB–CAR– PDMS) 50 and 30 μm.	N/A	N/A	LOD: 30-150 raw and treated water	GC-MS	Díaz/Ventura, 2002b

Table 2. Methods of analysis… (Continuation)

Compound	Sample preparation (extraction, elution, sample volume)	Derivatization	Internal standard/ surrogate standard	LOD and LOQ (ng/L), matrix	Detection	Reference
4-NP, 4-t-OP, BPA, E1, E2, E3, EE2	C_{18} cartridge; hexane dichloromethane (90:10), methanol-dichloromethane (90:10); Oasis HLB; methanol-diethylether (10:90); 1l sample	BSTFA	BPA-d_{16}	LOQ (HLB extraction): 2-10 in Milli-Q water	GC-MS	Jeannot *et al*., 2002
NP, NP1EO, NP2EO	LL extraction, pentane	N/A	$^{13}C_6$-NPnEO	LOD: 4-2122 in wastewater	HRGC/MS	Planas *et al*., 2002
t-NP, BPA, EE2	SPME 85 μm polyacrylate (PA), 100 μm polydimethyl siloxane (PDMS) and 65 μm polydimethylsiloxane /divinylbenzene (PDMS/DVB), 9.5 mL sample	N/A	4n-NP, β-estradioldiacetate, bromindanol and [$^2H_{14}$]BPA	LOD: 40-800 in wetland effluent	GC-MS	Braun *et al*., 2003
NP, NP1EO, NP2EO,NP3EO, NP1EC, NP2EC and others	Bond Elut C18-HF cartridge; methyl acetate, 500 mL	Chlorinated derivatives (ClNP, ClNP1EO and ClNP1EC) and brominated derivatives (BrNP, BrNP1EO and BrNP1EC)	OP-d, OP1EO-d, and OP1ECd as surrogates; phenanthrene-d_{10} and pyrened$_{10}$ as internal standards	LOD: 2.5-18 in pure water	GC/MS-MS	Hoai *et al*., 2003
4-n-NP, NP1EO, NP2EO,BPA, TCS	C-18, poly(divinylbenzene-co-N-vinylpyrrolidone), styrene-divinylbenzene e hydroxylated estyrene-divinylbenzene; dichloromethane-hexano (4:1); 100 mL simple	BSTFA and pyridine	BPA-d_{16}	LOD: 30-410, in wastewater LOQ: 110-1340, in wastewater	GC-MS	Gatidou *et al*., 2007
Paclobutrazol and myclobutanil enantiomers	AccuBond SPE ODS-C18 cartridges, 5 mL methanol, 200 ml tap water simple	N/A	N/A	LOD: 1500 LOQ: 2500	reversed-phase LC	Tian *et al*. 2010

Table 2. Methods of analysis… (Continuation)

Compound	Sample preparation (extraction, elution, sample volume)	Derivatization	Internal standard/ surrogate standard	LOD and LOQ (ng/L), matrix	Detection	Reference
			Pharmaceuticals			
Phthalates and parabens	(SPE) cartridges (500 mg C18, Supelco), 2.5 mL acetonitrile and 2.5 mL ethyl acetate LC-MS/MS , 5 mL acetone (GC-MS/MS), 500 mL and 1000 mL	mixture of 2mg dithioerytrol, 2ml trimethylsilylimidazole, and 1,000ml N-methyl-N-(trimethylsilyl)-trifluoroacetamide.	Isotope-labeled methyl paraben and phthalate metabolites	LOD: 1.0-8.4, pthtalates; 1.2-2.8, parabens	LC-MS/MS (Phthalates), LC-MS/MS and GC-MS/MS (Parabens)	Kusk *et al*. 2011
60 pharmaceuticals (carbamazepine, ibuprofen, diclofenac, ketoprofen, naproxen, and others)	RP-C_{18} cartridge, 4mL acetone, 1L sample; PPL Bond-Elut, 5 mL methanol, 1 L	PFBBr in cyclohexane with triethylamine	2,3-dichlorophenoxyacetic (2,3-D) as surrogate standard	LOQ: 13-32 in ground water	CG/MS	Sacher *et al*., 2001
Carbamazepine, ibuprofen, diclofenac, naproxen, clofibric acid and others)	Oasis HLB; ethyl acetate - acetone (50/50); 1L sample	Diazomethane	[$^{13}C_6$]metolachor, atrazine- d_3, MCPA-d_3, dimethenamide-d_3, Mecoprop- d_3, dihydrocarbamazepine	LOD: 1-10 in Drinking water, surface water and wastewater treatment plant effluent	CG-EM	Öllers *et al.*, 2001
95 organic wastewater contaminants	Oasis HLB and HLB cation exchange (MCX), CLLE; CH_3OH with NH_4OH, mixture of CH_3OH and $C_2HCl_3O_2$, CH_2Cl_2.	N/A	$^{13}C_6$-sulfamethazine, meclocycline, simatone $d_4$estradiol and $d_7$cholesterol as surrogate standard	Various	HPLC, LC/MS-ESI(+) using SIM, GC/MS	Kolpin *et al*., 2002
Nine acidic (pharamaceuticals)	Pressurized liquid extraction (PLE), HLB Oasis; methanol; 500 mL sample	N/A	N/A	NR	LC –ES-MS/MS	Miao *et al.*, 2002
Naproxen, ibuprofen, E1,E2, BPA, clorophene, triclosan, fluoxetine, clofibric acid, acetaminophen	SDB-XC Empore disk; methanol, dichloromethane and methane	BSTFA	Phenanthrene-d_{10} as internal standard; acetaminophen-d_4, BPA-d_{16}, and E1-d_4 as surrogate standard	LOD: 0.1-25.8 in surface water	CG-MS	Boyd *et al*., 2003
Diclofenac, ibuprofen, clofibric acid, phenazone, propyphenazone	C_{18} cartridge; 2.5mL methanol; 1L sample	200 µL PFBBr and 5 µL triethylamine in toluene, 110 ºC, 1h	3,4-D as surrogate standard; 2,4-dichlorobenzoic acid as internal standard	LOD: 38-340 in wastewater	GC/ITD-MS	Koutsouba *et al*., 2003

Table 2. Methods of analysis… (Continuation)

Compound	Sample preparation (extraction, elution, sample volume)	Derivatization	Internal standard/ surrogate standard	LOD and LOQ (ng/L), matrix	Detection	Reference
Ibuprofen, naproxen, ketoprofen, tolfenamic acid, diclofenac	Oasis HLB cartridge; ethyl acetate; 500 mL samples	MTBSTFA	Meclofenamic acid as surrogate standard PCB-30 as internal standard	LOQ: 20-50 in sewage water	GC-MS	Rodríguez *et al.*, 2003
Carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, naproxen	Waters oasis HLB; 1L Sample	Diazomethane	Dihydrocarbamazepine, Mecoprop-d3	NR	CG-MS	Tixier *et al.* , 2003
21 endocrine disrupting (phenols and acidic pharmaceuticals)	Oasis MAX SPE; methanol and formic acid in methanol (2:98)	EDCs by Pentafluoropropionic acid anhydride (PFPA); acidic pharmaceuticals by MTBSTFA	Deuterated E2, BPA for EDCs; 2,3-D for acidic drugs	LOD: 10-100 in wastewater	CG-MS	Lee *et al*., 2005
Acetaminophen, caffeine, carbamazepine, diclofenac, ibuprofeno, ketoprofen, naproxen,	Oasis HLB cartridges, methanol, acetonitrile and 50 mM KH_2PO_4 solution, 500 and 1000 mL	N/A	N/A	LOQ: 6.2-319.8 and 3.0-160 influent and effluent wastewater samples	HPLC-DAD	Santos *et al*., 2005
58 potential EDCs	Oasis HLB; methanol and methanol/MTBE (10:90), and DCM, 1 L	N/A	Related deuterated compounds	LOD: 1-10 in water	GC-MS/MS; LC-MS/MS	Trenholm *et al.* 2006
Clofibric acid, ibuprofen, carbamazepine, naproxen, ketoprofen, diclofenac	Oasis HLB; methanol	Tetrabutylammonium hydrogen sulphate ($TBAHSO_4$)	Deuterated chrysene	LOQ: 1.0-8.0 in drinking water	CG-MS	Lin *et al*., 2005
Ibuprofen and naproxen	Sodium dodecyl sulphate dodecil (SDS) hemimicelles formed onto orto-γ-alúmina, 0.3 M NaOH: methanol solution (70:30 v/v), 0.75-1 L samples.	N/A	N/A	NR	LC/UV	Costi *et al*. 2008
20 pharmaceuticals, 25 potential EDCs, and 6 other wastewater contaminants	SPE, Oasis HLB; methanol and methanol/MTBE (10:90), and DCM, 1 L	N/A	Related deuterated compounds	LOD: 1-10 in water	LC-MS/MS and GC-MS/MS	Benotti *et al*. 2009

Table 2. Methods of analysis… (Continuation)

Compound	Sample preparation (extraction, elution, sample volume)	Derivatization	Internal standard/ surrogate standard	LOD and LOQ (ng/L), matrix	Detection	Reference
Ibuprofen, naproxen, bezafibrate, diclofenac, ketoprofen	Oasis MCX 6-cm3 (150-mg) cartridges, 4 × 1 ml of acetone, 500 ml water sample	N/A	[$^{13}C_3$]-ibuprofen and [^{13}C-D_3]-naproxen	LOQ: 5-10 (surface water), 25-50 effluent WWTP	LC-MS/MS	Daneshvar *et al*. 2010
Ibuprofen, naproxen, ketoprofen, diclofenac, meclofenamic acid	C18 cartridges, 3x2mL of ethyl acetate, 100 mL wastewater sample	50 mL of BSTFA +1% TMCS with Pyridine (10 mL)	N/A	Wastewater: LOD, 0.37-3.1; LOQ, 1.1-10	GC-MS	Samaras *et al.* 2010
Diclofenac, furosemide, loratadine, metoprolol , nifedipine perindopril, salbutamol and others	Oasis-MCX cartridges, (3× 2 mL MeOH), 2mL (90/10 MTBE/MeOH), 2mL (2% ammonium, hydroxide in MeOH) and 2mL (0.2% NaOH in MeOH), 100-1000 mL surface water samples	N/A	Glibenclamide-d_{11}, atenolol-d_7, diclofenac-d_4, Amlodipine-d_4, simvastatin-d_6, chlorpheniramine-d_6 and hydroxybenzoic acid-d_6 as internal standard	LOD: 0.2-0.7 (method detection limit)	LC-MS/MS	Al-Odaini *et al*. 2011

APPI: atmospheric pressure photoionization
BPA: biphenil A
BrNP: Bromononylphenol
BrNP1EO: bromononylphenolmonoethoxylate
BrNP2EO: bromononylphenoldiethoxylate
CLLE: continuous liquid-liquid extraction
DAD: diode array detection
DES: diethylbestrol
DMC: dimethylcarbonate
E3: estriol
ECD: electron capture detector
EI: electronic impact
E1: estrona
E2: 17β-estradiol
EE2: 17α- ethinylestradiol
ESI: electrospray ionization
HFBA: heptafluorobutyric acid
HRGC: high-resolution gas chromatography
HPLC: high performance liquid chromatography
ITD: ion trap detector
LC-UV: liquid chromatography-uv detector
LL: liquid-liquid (extraction)
LOD: limit of detection
LOQ: limit of quantification
MBTFA: N-methyl-bis(trifluoroacetamide)
MSTFA N-methyl-N-(trimethylsilyl)trifluoroacetamide
MTBSTFA: *N*-Methyl-*N*-[tert-butyldimethyl-silyl]trifluoroacetimide
N/A: not applicable;
NCI: negative ion chemical ionization
NP: 4-nonylphenol
NP1EO: nonylphenol monoethoxylate
NP2EO: nonylphenol diethoxylate
NP3EO: nonylphenol triethoxylate
NP2EC: nonylphenoxy ethoxy acetic acid
NP1EC: 4-nonylphenoxy acetic acid
NR: not reported
PFBBr: pentafluorobenzyl bromide
SDB: styrene-divinylbenzene
SPME: solid phase microextraction
TBDMCS: tert-butyl dimethylchlorosilane
TFAA: trifluoroacetic anhydride
t-NP: technical-nonylphenol
4-n-NP: 4-n-nonylphenol
4-n-OP:4-n-octylphenol

3.1. Process O_3/pH↑

The action of ozone on organic material in aqueous media has been thoroughly studied; it has been concluded that the oxidation reaction occurs by molecular or radical routes (Beltrán, 1999). The molecular route acts selectively on organic compounds that have double bonds and are prevalent at low pHs. For the radical route, the principal oxidizing agent is the radical $HO^{\bullet}$ that acts in a non-selective form on organic compounds (Beltrán *et al*. 2012; Kim *et al*. 2011; Maniero *et al.* 2009; Beltrán *et al*., 2008, Beltrán, 2004, 1999; López-López *et al*, 2004; Hoigné/Bader, 1976).

The O_3 molecule and the $HO^{\bullet}$ radical have the potential for oxidation-reduction (E^o) of 2.8 and 2.07 volts, respectively; fluorine has a more elevated potential (3.0), and these are the three oxidant species with the highest potentials.

On the other hand, the action mechanisms of ozone on organic material present in water, by molecular and radical routes, show great differences in the magnitudes and velocities of reactions, the latter showing a function directly proportional to their respective constants, $k_{O3/M}$ y $k_{HO\bullet/M}$. Table 3 shows a comparison of the rate constants of ozone oxidation on some EDCs and pharmaceuticals (Rosenfeldt/Linden 2004; Vogna *et al*. 2004a; Andreozzi *et al*. 2003a; Huber *et al*. 2003; Andreozzi *et al*. 2002; Yao/Haag, 1991).

The difference between the magnitudes of the kinetic constants is caused by the action of the $HO^{\bullet}$ radicals originated during the decomposition of ozone. From this stem the importance of developing an AOP-O_3 and establishing the conditions under which the two-phase reactor (gas-liquid) must operate with the objective of achieving maximum efficiency in the production of $HO^{\bullet}$ radicals and consequently the degrading EDCs.

Some studies that used AOP-O_3 at different pHs and doses of oxidant to degrade different EDCs are presented in Table 4.

3.2. Process O_3/H_2O_2

Different studies have used AOP, particularly the O_3/H_2O_2 system, to degrade EDCs (Benitez *et al*. 2011; Maniero *et al*., 2009; Ikehata *et al*., 2006; Naghashkar/El-Din, 2005a, 2005b; Beltrán, 2004, 1999; Balcioglu/Otker, 2003; Zwiener/Frimmel 2000). During the application of the O_3/H_2O_2 system, the hydrogen peroxide (H_2O_2) hydrolyzes in the following manner:

- (1)

The mechanism for generation of $HO^{\bullet}$ radicals starts with a very rapid attack of ozone on the hydroperoxide anion (HO_2^-) which originated from the decomposition of H_2O_2 (Beltrán, 2004):

- - (2)

Tabla 3. Comparison of rate constants of molecular ($k_{O3}/_M$) and radical ($k_{HO\bullet/M}$) oxidation of O_3 on some EDCs and pharmaceuticals (adapted from various authors).

Sustancia	$k_{O3/M}$ ($L.mol^{-1}.s^{-1}$)	Reference	$k_{OH°/M}$ ($L.mol^{-1}.s^{-1}$)	Reference
Pentaclorofenol (phenol-chloride)	$> 10^5$	(1)	$4x10^9$	(1)
Atrazina (pesticide)	2.25-6	(1)	$(2.4\text{-}2.7)x10^9$	(1)
Endrin (pesticide)	<0.02	(1)	$1.1x10^9$	(1)
17α-etinilestradiol (ovulation inhibitor)	$7x10^5$	(2)	$1.08x10^{10}$	(3)
Diazepam (tranquilizer)	0.75	(2)	$7.2x10^5$	(2)
Benzafibrato (lipid regulador)	$5.9x10^2$	(2)	$7.4x10^9$	(2)
Carbamazepine (antiepileptic/analgesic)	$7.81x10^4$	(4)	$2.05x10^9$	(5)
Paracetamol (analgesic)	$4.29x10^4$	(6)	$2.2x10^9$	(6)

(1): Yao/Haag 1991 (3): Rosenfeldt/Linden 2004 (5): Vogna et al. 2004[a]
(2): Huber et al. 2003 (4): Andreozzi et al. 2002 (6): Andreozzi et al. 2003a

Benitez *et al*. 2011; Maniero *et al*., (2009), Ikehata *et al*., (2006); Naghashkar/El-Din (2005a, 2005b) have shown the effectiveness of AOPs for degrading a great variety of EDCs (natural and synthetic hormones and pharmaceuticals) at a laboratory level, highlighting the advantages of coupling O_3/H_2O_2 as a simple, effective and economical process with respect to other AOPs.

3.3. Process O_3/Cat

This O_3/Cat [Cat=Co(II), Fe(II), Mn(II), Ti(II)] type of AOPs, also called catalytic ozonation, is investigated for the production of $HO^{\bullet}$ radicals to degrade EDCs and, in general, recalcitrant contaminants such as pesticides, dyes and chlorinated compounds, among others (Yang *et al*. 2009; Sharma, 2008; Ikehata *et al*., 2007; Beltrán, 2004; Rivas *et al*., 2003; Cortés *et al*., 2000, Gracia *et al*., 2000). In this sense, Rivas *et al*. (2003) have

shown that the decomposition of ozone in the presence of Co(II), follows a pseudo-first-order kinetic with respect to ozone. These authors have also established that the generation of $HO^{\bullet}$ radicals, from the catalysis of O_3 by Co(II), occurs in an acidic medium; this mechanism is represented by Equation 3. However, this mechanism can be inhibited by alkaline media. This phenomenon is attributed to the f act that $Co(OH)_2$ at pH>8 is less soluble, which can lead to the precipitation of the latter, causing diminished catalyzing power of Co(II).

(3)

Cortés *et al*. (2000) successfully applied the O_3/Cat AOPs, at a laboratory level and in a discontinuous regime, to degrade organochlorine compounds at concentrations of $6x10^{-5}$ M; utilizing Fe(II) and Mn(II) as catalysts at concentrations of $6x10^{-5}$ M. In the first stage, the O_3/Fe(II) y O_3/Mn(II) AOPs were applied to prepared solutions with chlorinated compounds; in a second stage this AOPs was applied to industrial waters also containing chlorinated compounds. The results of the first stage showed an oxidation efficiency of these compounds on the order of 98 and 100 per cent. In the second stage, Cortés *et al*., (2000), established that the degradation velocity of the chlorinated compounds is a function inversely proportional to the number of Cl^{-1} ions present in the compound, given that chlorobenzene was more rapidly oxidized than di-, tri-, tetra- and pentachlorobenzene. This also shows that the chemical stability and the recalcitrance of the compound are due to the number of chlorines present in the organic chemical species.

Ikehata *et al*. (2007) and Beltrán (2004) have applied O_3/Cat AOP to sources of drinking water supply, showing degradation of EDCs of more than 80 per cent, in relatively short times, following a first order kinetic in the presence of Co(II) and Mn(II). Also, these authors have shown that the $HO^{\bullet}$ radicals, generated during the destruction of O_3, play an important role in the oxidation of EDCs.

Table 4 presents a compilation of AOP-O_3 as described above. The references are listed in chronological order from the beginning of the present decade, without considering the type of EDCs or recalcitrant compound.

Tabla 4. AOP-O3 applied to different types of water and under different conditions

(Adapted from Esplugas et al., 2007)

Compound	Type of water	Treatment	Operation Conditions	Results and Commentaries	Reference
Clofibric acid, ibuprofen and diclofenac	Distilled and drinking water	O_3/H_2O_2 molar ratio (O_3/H_2O_2) = 2:1:1 Tr = 10 min	= 1.0 mg L^{-1} C_0= 2 μgL^{-1}, distilled water = 1.0 mg L^{-1} C_0= 2 μgL^{-1}, distilled water = 1.0 mg L^{-1} C_0 = 2 μgL^{-1}, drinking water = 3.7 mg L^{-1} C_0= 2 μgL^{-1}, drinking water = 5.0 mg L^{-1} C_0= 2 μgL^{-1}, drinking water	8% of clofibric acid, 12% of ibuprofen and 97% of diclofenac were removed 50% of clofibric acid, 50% of ibuprofen and 100% of diclofenac were removed 10% of clofibric acid, 30% of ibuprofen and 100% of diclofenac were removed 90% of clofibric acid, 90% of ibuprofen and 100% of diclofenac were removed 97.9% of clofibric acid, 99.4% of ibuprofen and 100% of diclofenac were removed	Zwiener /Frimmel, 2000
Carbamazepine	Aqueous solution	Ozonation	Ratio O_3/CBZ = 10; C_0 = 0.8 mg L^{-1}; = 1.0 mg L^{-1}; Ratio O_3/CBZ = 1.0 (consumption); C_0= 118 mg L^{-1}; Tr = 10–60 min	Complete removal of carbamazepine in natural water was reached. After 60 min of treatment a little TOC removal was observed	Andreozzi *et al*., 2002
Carbamazepine, benzafibrate, diclofenac and clofibric acid	Distilled and drinking water	Ozonation	C_0 =1 μgL^{-1}; = 0.5 − 3.0 mg L^{-1}; Tr=20 min	97% of carbamazepine and diclofenac were eliminated with ozone dose of 0.5 mg L^{-1}, only 10–15% removal of clofibric acid with the same dose. Bezafibrate was 50% removed with ozone dose of 1.0–1.5 mg L^{-1} and 80% was removed with ozone doses 3.0 mg L^{-1}. At higher ozone dose (2.5–3.0 mg L^{-1}) 40% of clofibric acid was removed	Ternes *et al*., 2002
Paracetamol	Aqueous solution	Ozonation	pH 2.0 and 7.0; C_0 = 5.0 mM; T=25 ◦C	Complete removal of paracetamol with 30% mineralization. Oxalic, glioxalic, cetomalonic and formic acids and hydroquinone were identified as intermediates	Andreozzi *et al*., 2003a
Clofibric acid	Aqueous solution	Ozonation	Tr = 60 min; pH=2.0–6.0; C_0 = 1.0–1.5 mM; aqueous = 1.0×10−5 mol L^{-1}	100% removal of clofibric acid was reached in 20 min with 34% mineralization. 49% mineralization was reached in 60 min. No halogens compounds were detected in the oxidation product	Andreozzi *et al*., 2003b
Bezafibrate, carbamazepine, diazepam, diclofenac, 17β-estradiol, 17α-ethinylestradiol, ibuprofen, iopromide, sulfamethoxazole and roxithromycin	Milli-Q, river and lake water	Ozonation	= 0.1; 0.2; 0.5; 1.0 and 2.0 mg L^{-1}; C_0 (benzafibrate)= 1 μM, C_0 (EE2)= 4 μM ; natural water properties: pH 7.2–7.9; COD= 0.8–3.7 mg L^{-1}; alkalinity = 0.7–5.8 M HCO^{3-}	Ozone doses ranging from 0.2 up 0.5 mg L−1 were observed with 97% removal of all compounds. Removal of bezafibrate was lower.	Huber *et al*., 2003

Tabla 4. AOP-O3 applied… (Continuation)

Compound	Type of water	Treatment	Operation Conditions	Results and Commentaries	Reference
Iodinated X-ray contrast media, antibiotics, betablockers, antiphlogistics, lipid regulator metabolites, antiepileptics and estrogens	STP effluent	Ozonation	= 5, 10, 15 mg L^{-1}; effluent properties: pH 7.2; DOC=23 mg L^{-1}; COD=30 mg L^{-1}; SST = 4.5 mg L^{-1}	Ozone doses ranging from 5 up to 15mg L^{-1} were necessary for complete removal of these compounds; were reduced below the LOQ	Ternes *et al.*, 2003
Estrogens (17β-estradiol and 17α-ethinylestradiol) and bisphenol A	Distilled water	Ozonation	C_0 = 0.1μM; *T*=20 ◦C; contact time = 1–120 min; = 1.5 mg L^{-1}	Exception for BPA, all samples were oxidized to below detection levels. A reduction of estrogenic activity was reached.	Alum *et al.*, 2004
Diclofenac	Distilled water	Ozonation	pH 5.0; 5.5 and 6.0; scavenger = *tert*-butyl alcohol; C_0 = 0.1 mM; aqueous = 0.05 mM	100% of chlorine release was observed and 32% mineralization	Vogna *et al.*, 2004b
Natural estrogen (17α-estradiol)	Distilled water	Ozonation	C_0 = 5.2 μM; *T*=20 ◦C; contact time 30 min; = 5.0 − 15 mg L^{-1}; pH 6.0; experiments with and without fluvic acid	99% removal of 17β-estradiol with ozone dose of 5mg L^{-1} in 15 min or ozone dose of 15 mg L^{-1} in 4min. It was observed a reduction of estrogenic activity.	Kim *et al.* 2004
Synthetic estrogen (17α-ethinylestradiol)	Milli-Q purified water	Ozonation	C_0 = 10 μM, pH 8; = 5 a 24 μM; C_0 = 1 μM, = 50 a 100 μM	Oxidation products formed during the ozonation of EE2 was identified. Ozone doses ranging from 0.5 up to 10 mg L−1 removed estrogenicity.	Huber *et al.*, 2004
Antibiotic (amoxicillin)	Aqueous solution	Ozonation	C_0= 0.5 mM; = 0.16 mM, pH 2,5-5,0	Low mineralization and some by-products were identified.	Andreozzi *et al.*, 2005
Natural estrogen (17β-estradiol)	Milli-Q and drinking water	Ozonation	C_0 = 10 and 50 μg L^{-1}, pH 3.7 and 11; = 0.5 a 10 mg L^{-1}	Ozonation was able to promote extensive degradation of 17β-estradiol and to reduce its estrogenic activity. Removal of 99.6% was achieved with ozone dose of 10 mg L^{-1}. At pH 7 and 11 the estrogenic activity was not completely removed, even with an increase of the dosage of ozone.	Bila *et al.*, 2005
Natural estrogen (17β-estradiol) and bisphenol (bisphenol A)	Aqueous solution	Ozonation	C_0 = 0,10 mM; = 7.5-16 μM	The reaction between bisphenol A and ozone is slower than the reaction between 17β-estradiol and ozone.	Irmak *et al.*, 2005

Tabla 4. AOP-O_3 applied … (Continuation)

Compound	Type of water	Treatment	Operation Conditions	Results and Commentaries	Reference
Pesticide (atrazine) pharmaceuticals (carbamazepine)	Drinking water	Ozonation	pH 7.5; = 1.5-2.0 mg L^{-1}	High efficiency in removing micropollutants using ozonation after filtration and coagulation/flocculation and is highly influential in the fate of these compounds in drinking water treatment regardless of the seasonal time frame.	Hua *et al*., 2006
Antibiotic (clarithromycin)	Milli-Q water	Ozonation	C_0 = 0.1 mM; = 10 μM, T = 20 ºC	Biological activity (inactivation drugs) of clarithromycin was reduced after ozonation	Lange *et al*., 2006
Pesticides (alachlor, atrazine, chlorfenvinphos, isoproturon, diuron)	Distilled water	Ozonation	C_0 = 16-20 mg L^{-1} = 26.8g m^{-3}	Large amounts of ozone were spent to remove pesticides. Complete removal of TOC was hard to achieve.	Maldonado *et al*., 2006
Benzafibrate (lipid regulator)	Distilled water	Ozonation	C_0 = 0.2-0.5 μM, pH 6 to 8 = 1 μM	The complete BZF abatement is achieved. However, only a small part of the substrate is mineralized. Second-order kinetic constants for the substrate were found: 0.27-1.0 x 10^4 M^{-1} s^{-1} (absolute method) and 0.15-1.3 x 10^4 M^{-1} s^{-1} (competitive method).	Dantas *et al*., 2007
Ibuprofen, bezafibrate, amoxicillin, sulfamethoxazole	Pure water	Ozonation	C_0= 10 μM = 1 mM	In the ozone-membrane filtration hybrid experiments, the pre-ozonation was able to reduce the membrane fouling two-fold lower than process alone.	Soo Oh *et al*., 2007
Steroids (17β-estradiol 17α-ethinylestradiol)	Milli-Q water	Ozonation O_3/H_2O_2	C_0= 10 μg L^{-1} (17β-estradiol) C_0= 10 μg L^{-1} (17α–ethinylestradiol) Relation 2:1 O_3/H_2O_2	99.7% and 98.8% were removed at pH 11 separately while 100% and 99,5% were eliminated at pH 3 for 17β-estradiol and 17α-ethinylestradiol, respectively Total estrogenic activity was removed at pH 3 for ozonation o O_3/H_2O_2 process	Maniero *et al*., 2009
Bisphenol A	Milli-Q water	Ozonation	C_0=100 μM in 100 mL =15-400 μM	Reaction subproducts were analyzed and identificated: catechol, ortoquinone, muconic acid, benzoquinone y 2-(4-hydroxyphenyl)-propan-2-ol	Deborde *et al*., 2008
Naproxen and carbamazepine	Distilled water	Catalytic ozonation	C_0=6.51x10^{-5} M (naproxen) C_0= 6.35 x10^{-5} M (carbamazepine) =0.63-0.83 mM pH=3-7 T=25 °C	The catalyst promotes the decomposition of ozone under acidic conditions, while at neutral pH it behaved as an inhibitor of the ozone decomposition in pure water. The catalyst promotes mineralization in slightly acidic conditions, a result linked to the adsorption of reaction intermediates on acid catalytic sites.	Rosal *et al*. 2008
Phenazone, ibuprofen, diphenhydramine, phenytoin, diclofenac	Distilled water	Catalytic Ozonation	C_0=3 mg L^{-1} =30 mg L^{-1} MnO*x* supported by mesoporous alumina (MnO*x*/MA)	MnO_x catalyst allows formation and activation of surfaces hydroxil groups; it produced a high catalytic reactivity. Catalyst was highly effective in pharmaceuticals degradation in aqueous solution	Yang *et al*., 2009

Tabla 4. AOP-O_3 applied … (Continuation)

Acetaminophen, carbamazepine, diclofenac, naproxen and others	Effluent WWTP	Ozonation	pH: 6.5 - 6.8, DOC= 2.7 - 3.4 mg L−1, UV_{254}= 0.0514 cm^{-1} to 0.0779 cm^{-1}. C_0=2-402 mg L^{-1} =2, 4, 6 mg/L	The removal efficiency increased as the O_3 dose increased. Twenty-five PPCPs, including carbamazepine, crotamiton, and diclofenac, were removed by>90% at the lowest O_3 dose. Ten of the 11 antibiotics had removal efficiencies of at least 90%, irrespective of O_3 dose.	Kim *et al.* 2011
Metoprolol, naproxen, amoxicillin phenacetin	Ultrapure water, groundwater and surface water	Ozonation O3/H2O2	Water properties: pH=7.6-8.1, TOC (mg L−1) = 1-6 22.5 COD (mg L−1) = 1.9-36.3 Conductivity (□S cm−1) =128-1566 C0= 1 □M, =0.3 □M = 10 □M	The pseudo-first-order rate constants obtained in the oxidation of metoprolol, naproxen, amoxicillin, and phenacetin, in real water matrices, were 0.128, 0.346, 0.176, 0.177 m-1. The combination of O3/H2O2 provided higher rate constants than the single ozonation in the water matrices tested. The degradation rate was higher in the UP water than in the secondary effluents, as a consequence of the DOM present, which consumes part of the oxidant in the oxidation of other substances present.	
Diclofenac, sulfamethoxazole and caffeine	Ultrapure water and effluent WWTP	Ozonation	Effluent properties: pH 7–8, TOC: 35 mg L^{-1}, IC: 23 mg L^{-1}, COD: 58–84 mg L^{-1}, BOD: 30–60 mg L^{-1} C_0=10 mg L^{-1} = 2, 4, 6 mg/L	By comparing the experiments of single ozonation with and without the presence of bicarbonate/carbonates no significant differences were observed regardless of the pharmaceutical compound treated; therefore direct ozonation is the predominant mechanism of DCF and SMT removal. For all compounds, removal rates are something slower than in ultrapure water, especially in the case of DCF although the reaction time needed to reach 99% conversion is similar.	Beltrán *et al*. 2012

BOD: biochemical oxygen demand
COD: chemical oxygen demand
DOC: dissolved organic carbon
IC: inorganic carbon
STP: sewage treatment plant
TOC: total organic carbon
UV_{254}: UV absorbance at 254 nm
WWTP: wastewater water treatment plant

According to the information in Table 4, ozonization is the AOPs most used for the removal of EDCs and ECs. Approximately 90 per cent of AOPs encountered in the literature corresponds to ozonation, ozonation with H_2O_2 and ozonation with a catalyst. The removal of EDCs and ECs was achieved using doses of ozone of 0.1 to 40 mg L^{-1}. Removals of approximately 90% were achieved for the following EDCs: pesticides, anti-inflammatories, anti-epileptics, antibiotics and natural and artificial estrogens. However, some substances are more recalcitrant to oxidation with ozone, such as *clofibric acid, ibuprofen* and *X-ray contrast media agents*.

Conclusions

The EDCs are a group of compounds which are potentially dangerous for the endocrine system of living beings, found in the environment, particularly in surface water, in very low concentrations, complicating their identification. Another group, the ECs, which includes steroids (natural and synthetic), pharmaceutical, veterinary drugs, antiseptics and personal care products is also found in water. Both EDCs and ECs affect water quality and potentially impact sources of drinking water, ecosystems and human health. The potential disruption effects of ECs on living things are still uncertain and require further investigation.

The need for identification and analysis of EDCs has caused the development of analysis methods that use tools like *Gas Chromatography* (GC) or *Liquid Chromatography* (LC) coupled with *Mass Spectrometry* (MS) detection; the most commonly used are GC/MS, GC/MS/MS in tandem, and LC/MS/MS. The use of the solid phase extraction technique reduces time and resources compared to conventional methods.

There are a great number of *advanced oxidation processes* (AOPs) that are being investigated on a laboratory level for degrading EDCs and recalcitrant or poorly biodegradable organic material in general. However, AOP-O_3 are the most studied of high-efficiency processes (higher than 80 per cent) obtained in relatively short times - within minutes. In addition, the AOP-O_3 has lower costs with respect to other AOP in the generation of $HO^{\bullet}$ radicals. AOP-O_3 are capable of achieving partial or total oxidation (mineralization) of the organic material; the level of oxidation diminishes the possibility of forming by-product compounds that are harmful to health. It should be mentioned that the times and concentrations of ozone necessary for the oxidation of the EDCs change according to the nature of the compound.

Finally, the use of the AOP-O_3 promises to be one of the most appropriate technological resources not only for the treatment of surface waters containing EDCs, including hormones and

pharmaceuticals, but in general for industrial effluents contaminated with compounds of low biodegradability such as dyes, lignins from paper mill effluents, phenolic compounds, detergents, organochlorine compounds and personal care products. Despite the effectiveness of the AOP-O_3, it is necessary to perform a technical evaluation of cost and benefits to verify that AOP-O_3 are technologically and economically viable.

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CHAPTER 2

OPTIMIZATION OF ANALYTICAL CONDITIONS TO DETERMINE STEROIDS AND PHARMACEUTICALS DRUGS IN WATER SAMPLES USING SOLID PHASE-EXTRACTION AND HPLC

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Abstract

Two reliable methods were optimized to determine two steroids (17β-Estradiol and 17α-Ethinylestradiol) and two pharmaceutical drugs (ibuprofen and naproxen) using Solid-Phase Extraction (SPE) for sample preparation and High Performance Liquid Chromatography (HPLC) for analysis. SPE (C_{18}) conditions were evaluated varying elution solvent volume, pH conditions and sample mass in the cartridge and reduction techniques of the extract. The efficiency of the analytical methods was evaluated by spiking ultrapure water samples with compounds at three and four levels of concentration for steroids and pharmaceutical drugs, respectively. The recoveries were independent (p>0.05) of added mass of target analytes with a repeatability lower than 6.5% for steroids and 12.1% for pharmaceuticals compounds. The recovery factor (coefficient of variation, CV) was higher than 83% for steroids (CV < 3.8 %) and > 93 % for pharmaceuticals (CV< 5.2 %). The optimized analytical method was applied for the evaluation of a steroid degradation test using ozone, finding that the estimated limit of detection is sufficient to determine the residual mass (μg L^{-1}) of 17β-Estradiol after the experiment.

1. Introduction

The ever increasing use of synthetic steroids and pharmaceutical products both in humans as well as in animals is becoming a new environmental issue to such an extent that the interest in collecting information regarding the origin of these substances in the environment and their possible effects on humans and ecological systems has increased (EEA, 2010; Kümmerer, 2010). So far, the generalized use of oral contraceptives formulated with steroids capable of inducing estrogenic responses in fishes at low concentrations such as 1 ng L^{-1}, has raised concern among the scientific community due to the potentially dangerous consequences on the aquatic medium (Vadja *et al.*, 2008; Fent *et al.*, 2006).

The environment, specifically aquatic, presents diverse pathways for contamination by pharmaceuticals and steroids. Such compounds enter the sewage system through urine and feces and finally end up in wastewater treatment plants (WWTPs) or are discharged directly into bodies of surfaces water. The uses of residual sludge from WWTPs that

contain estrogens in agriculture are another source of contamination in surface waters through run-offs (Gomes *et al*. 2011; Casey *et al*. 2003). Also, the WWTPs' effluents that originate from the pharmaceutical industry are an important source responsible for the pharmaceuticals present in the environment (Rodriguez *et al*. 2003). The low efficiencies of the WWTPs to degrade two classes of compounds due to their persistence and also effluents discharged from urban and industrial sources without any previous treatment result in the presence of natural and synthetic estrogens in surface water (Gomes *et al*., 2011; Peng *et al*. 2008).

Pharmaceutical drugs occurring in wastewaters or WWTP effluents have been reported in several research papers in concentration levels of μg L^{-1} for naproxen and ibuprofen (Samaras *et al*., 2010; Radjenović *et al*., 2009). For steroids, specifically natural ones and the synthetic steroids considered to be the most powerful estrogenic compounds, several studies have also reported very low environmental concentrations on the order of μg L^{-1} (Benotti *et al*., 2010; Jafari *et al*., 2009; Chen *et al*. 2007). Since these substances represent a potential danger for human health and their environmental levels, it is important to monitor both pharmaceuticals as well as steroids in water. Therefore, robust and reliable analytical methods are required which will allow estimating the presence of such compounds in water samples (Samaras *et al*., 2010; Santos *et al*., 2005; López de Alda/Barcelò, 2000). Although several authors have determined steroid concentration in water by using gas chromatography coupled to mass spectrometry (GC-MS), using solid-phase extraction as sample preparation method (Gibson *et al*., 2007; Liu *et al*. 2009), HPLC coupled to mass spectrometry (HPLC-MS) has also been used by other authors (Miège *et al*. 2009; Koh *et al*. 2007; Almeida/Nogueira, 2006). It is important to highlight that these techniques are robust, but at the same time costly, which does not allow many laboratories to acquire such infrastructure to develop similar methodologies. Considering the fact that most of them have High-Performance Liquid Chromatographs with Diode-Array Detection (HPLC-DAD), the implementation of analytical methods to determine pharmaceuticals and steroids in water by using this available analytical infrastructure is then justified. Before chromatography gas or liquid analysis, sample preparation techniques must be carefully selected and optimized because the low concentration of steroids and pharmaceutical drugs (López de Alda/Barcelò, 2000) can make the detection difficult in environmental samples

but the determination is becoming even more challenging when target analytes are degraded and then their concentration are as low that analytical signal become undetectable reliably in treated effluent. The solid-phase extraction (SPE) is one of the alternatives more frequently used with this purpose (Thurman/Mills, 1998), since it isolates the analytes from liquid sample and then these are concentrated (Jakobsen *et al*. 2003). In spite of there are so many reports on the analysis of steroids and pharmaceutically drugs (Samaras *et al*. 2010; Chen *et al*. 2007; Rodriguez *et al*. 2003; López de Alda/Barcelò, 2000), these are more as a guide or alternative method, therefore their conditions still must be optimized before be applied on samples and a step implementation and evaluation of quality analytical parameters in laboratory is necessary.

The objective of this chapter was the optimization of analytical conditions for the extraction and determination of two steroids and two pharmaceuticals compounds by SPE and subsequences analysis HPLC-DAD in water ultrapure samples, with the aim to evaluate the efficiency of their chemical degradation by using ozone processes. The optimized method includes the extraction and determination of sodium phenolate, which is the reagent used in competitive kinetics implemented in the experimental stage (Chapter 3).

2. Material and Methods

2.1. Chemicals and Materials

The compounds utilized in this work were selected according to their commercial demand, frequency of use, and presence in bodies of water. These were two steroids: one natural, 17β-Estradiol (E_2), and one synthetic, 17α-Ethinylestradiol (EE_2) (Jafari *et al*. 2009; Benotti *et al*. 2010); and two anti-inflammatory pharmaceuticals, naproxen and ibuprofen (Samaras *et al*. 2010; Radjenović *et al*., 2009, Santos *et al*. 2005). The standards of compounds (purity in parenthesis) of E_2 (98 %), EE_2 (98%), naproxen and ibuprofen (98%) were purchased as powders from Sigma-Aldrich (St. Louis, MO). The stock standard and further working solutions were prepared in methanol. The solvents, HPLC grade, were obtained as follow: acetonitrile (ACN) was from Tedia (Fairfield, OH), methanol (MeOH from J.T. Baker (Phillipsburg, NJ) and ultrapure water was obtained directly in our laboratory from an ultrapure water system (Nanopure, Barnestead). The packed syringe barrels with 500 mg of C_{18} Strata® (d_p 55 μm) SPE was acquired from Phenomenex

(Torrance, CA). The potassium phosphate monobasic (KH_2PO_4) and sodium phenolate with 99% purity was acquired from Sigma (St. Louis, MO).

2.2. Preparation of Samples and Standard Solution Stock

The samples were prepared by spiking ultrapure water with a known amount of the selected compounds. The optimization and adjustment of conditions procedure were carried out at only one concentration level, while evaluation of method was done at different levels; three for steroids and four for pharmaceuticals compounds, which were based on the levels reported in the literature (Santos *et al*., 2005; López de Alda/Barcelò, 2000). Although, only one type of stationary phase was used, the samples spiked with steroids and pharmaceuticals were processed separately because these compounds have different chemical properties. The standard stock solution of 17β-Estradiol (E_2) and 17α-Ethinylestradiol (EE_2) (2500 μg mL^{-1}) was prepared by dissolving 125 mg of both compound in 50 ml of methanol in volumetric flask. The working standards solutions (0.5 – 50 μg mL^{-1}) were prepared by diluting the stock solution with methanol. For naproxen and ibuprofen (500 μg mL^{-1}) the stock solution was prepared by dissolving 25 mg of both compounds. The working standard solution for naproxen (0.10 – 5.0 μg mL^{-1}) and ibuprofen (1-250 μg mL^{-1}) were prepared from the stock solutions with methanol. The stock solution of 100 mg L^{-1} of sodium phenolate was prepared with methanol and working standard solutions (0.5- 10 μg mL^{-1}) were prepared by diluting aliquots of stock solution in the same solvent.

2.3. Optimization and Evaluation of Analytical Procedure

2.3.1. Conditioning of Solid Phase

The C_{18} cartridges were conditioned before the extraction step using methanol, ethyl acetate, acetonitrile and water. For each kind of compound a different sequence was used. For steroid extraction, conditioning was done by passing 8 mL of acetonitrile through of the phase, then 7 mL of methanol and finally 5 mL of water. For the pharmaceuticals, 3 mL of ethyl acetate, and then 3 mL of methanol and 3 mL of water were used. Both sequences of solvent were based on previous work (Santos *et al*., 2005; López de Alda/Barcelò, 2000),

respectively. For sodium phenolate, the cartridge C_{18} was conditioned with the same sequence used for drugs.

2.3.2. Efficiency of the Eluting and Volume Solvent

The capacity and effectiveness of elution solvent from the C_{18} cartridges were tested by using the selected solvents, acetonitrile for the steroids and ethyl acetate for the pharmaceuticals and sodium phenolate. In separate experiments, both solvents were spiked with known amounts of the compounds and were passed by gravity through a column that was preconditioned. Later, seven aliquots of 2 mL were collected and then analyzed separately to known total recovery and volume necessary for the elution step. For steroid a solution of 5 mg L^{-1} was used and for pharmaceuticals and sodium phenolate solutions of 8.3 mg L^{-1} (naproxen), 83.3 mg L^{-1}, (ibuprofen) and 5.50 mg L^{-1} (sodium phenolate) were used. The amounts of compounds that were selected were based on environmental levels previously reported (Santos *et al.*, 2005; López de Alda/Barcelò, 2000).

2.3.3. Solid Phase Extraction Conditions

The spiked ultrapure water samples were passed through the conditioned cartridges placed in a Manifold Varian VAC ELUT-20, which was connected to a vacuum pump with a pressure and vacuum controller. For steroids the flow rate of 3.5 mL min^{-1} and a vacuum pressure of 3 in Hg, while for the pharmaceuticals and sodium phenolate these were 4.2 mL min-^{1} and 4.5 in Hg. Before the elution step, the cartridges were dried under vacuum (5 in Hg) for approximately twenty minutes to remove all water because these molecules could make the elution defaulted and yield low recoveries of compound from the stationary phase. The elution of the steroids was done with two aliquots (5 mL) of ACN and for pharmaceuticals and sodium phenolate it was carried out with three aliquots (1 mL) of ethyl acetate and combined in an amber bottle.

2.3.4. Effect of the pH on the Solid Phase Extraction of Pharmaceutical Compounds

The efficiency of extraction for the pharmaceuticals was evaluated at neutral (pH=7.0) and at acid conditions (pH=4.5) with the objective to know the effect on recovery. Both pH of the water sample and that used for conditioning of the stationary phase was

fixed with a buffer solution (KH_2PO_4 50 mM). For steroids and sodium phenolate, was evaluate only neutral pH for extraction recovery.

2.3.5. Efficiency of the Reduction Technique

Two different methods were used to evaluate the efficiency of the reduction technique. One method consisted of subjecting the extracts to a gentle stream of chromatographic nitrogen gas and for the others the extract were reduced using a Büchi R-210 rotary evaporator (30 rpm) at 40 °C, with vacuum pressure (5 in Hg). All reduced extracts were adjusted to 1 mL.

2.4. Evaluation of the Optimized Analytical Conditions

To check recoveries, linearity, precision and limit of detection (LOD) of the optimized methods, known amounts of each compound at different concentration levels were added to ultrapure water (1 L). Therefore, three concentrations levels were used for both steroids (12.5, 25, 50 μg mL^{-1}), fourth levels for naproxen (2.5, 6.25, 12.5, 25 μg mL^{-1}) and ibuprofen (25, 62.5, 125, 250 μg mL^{-1}), and three levels for sodium phenolate (2.0, 5.3 and 6.6 μg mL^{-1}). Each spiked level was assayed in duplicate using optimized conditions in each step of the analytical method.

2.5. Chromatography Analysis

2.5.1. Apparatus and Conditions

The determination for all compounds was performed on HPLC-DAD Varian ProStar 7725 equipped with Varian ProStar 230 DAD detector. The mobile phase for steroids separation was prepared by mixing acetonitrile and ultrapure water in a gradient elution. For pharmaceuticals, this was prepared by mixing methanol and 50 mM potassium dihydrogen phosphate buffer. The mobile phases were filtered through 0.45 μm nylon filters (Millipore) and degassed before use. The chromatographic separations were performed using LiChrospher 100 RP-18, 5 μm, 250 mm × 4.6 mm i.d. column (Agilent Technologies, Waldbronn, Germany) for all compounds, eluted with the mobile phase at the flow rate of 1.0 $mL \cdot min^{-1}$. The measurements were made with an injection volume of 100 μL for steroids and 20 μL for pharmaceuticals. The detection was carried out with a

Varian ProStar 230 (Walnut Creek, CA); diode-array detector (DAD); using the maximum wave-length (λmax) of 197 nm for steroids and 220 and 230 nm for ibuprofen and naproxen, respectively. The maximum wave-length for sodium phenolate was 197 nm (Figure 1).

2.5.2. Linearity

The linearity of the HPLC-DAD analysis was checked by analyzing seven solutions in the range of 1.0-50 μg mL^{-1} for steroids and 0.5-50 μg mL^{-1} and 0.5-250 μg mL^{-1} for naproxen and ibuprofen, respectively. For sodium phenolate, six solutions were used in the range of 0.5-10 μg mL^{-1}. Each concentration was analyzed in triplicate. Calibration curves for each compound were generated by plotting the analyte peak area against theoretical concentration of the analytes. The linear regression coefficient (r) for each compound were higher than 0.998.

2.5.3. Limits of Detection and Quantification

Limits of detection (LOD) and quantification (LOQ) were determined in accordance with Mitra (2003). The LODs were 0.65 μg mL^{-1} and 0.57 μg mL^{-1} for EE_2 and E_2, respectively, and LOQ values for EE_2 and E_2 were 2.16 μg mL^{-1} and 1.89 μg mL^{-1}, respectively. Similarly, for pharmaceutical compounds the LODs were 0.20 μg mL^{-1} (naproxen) and 0.89 μg mL^{-1} (ibuprofen) and LOQs were 0.65 μg mL^{-1} (naproxen) and 2.97 μg·mL^{-1} (ibuprofen), respectively. The LOD and LOQ for sodium phenolate were 1.00 μg mL^{-1} and 3.33 μg mL^{-1}, respectively.

2.5.4. Specificity

The specificity was evaluated for interference at the retention times of E_2, EE_2, naproxen, ibuprofen and sodium phenolate. Lack of interfering peaks in the spiked water sample with all compounds at the retention times of the five compounds was taken as an indication of the specificity of the methods (Figure 1).

2.6. Application of method

The analytical method was applied to evaluate the efficiency of degradation of E_2 by using ozone. The tests were carried out in batch reactors adapted with 100 mL flasks, in analyte concentrations prepared in ultrapure water of 0.7 μM with ozone doses of 1.3 μM from stock solutions. The assays were carried out in triplicate at a pH=6 and at 21°C (Huber *et al*. 2003). The ozone was generated by a Pacific Ozone G11 ozone generator (Benicia, CA, USA) equipment and ultrapure water saturation by bubbling into a Pyrex glass reactor of 2 L per batch at 20°C and pH=6, obtaining stock solutions saturated with ozone, 10 mg L^{-1} (0.20 mM). Ozone concentrations and residual ozone were measured using the indigo colorimetric method (Clesceri *et al*. 1998). One aliquot from stock solution (500 μL) was taken and added to the flask that contained the E_2; later, the flask was vigorously agitated during 3 s; after one minute of reaction, the residual ozone was zero (by reaction).

3. Results and Discussion

3.1. Elution Conditions

Initially, it was found that the compounds were eluted efficiently from the stationary solid phase (0.5 g of C_{18}) with recoveries of 90% for E_2 and 82 % for EE_2 when 10 mL of ACN were used; 94 % of naproxen and 85 % of ibuprofen with 3 mL of ethyl acetate; and no more 45% of sodium phenolate with 3 mL of ethyl acetate. Those results were obtained using standard solutions that contained 50 μg of each one of the steroids, 83 μg and 833 μg of naproxen and ibuprofen, respectively, and 55 μg of sodium phenolate.

3.2. Effect of pH on Extraction Procedure

The results showed that the recoveries of pharmaceutical compound increased with a reduction in pH; with a pH equal to 7.0 the recoveries for naproxen and ibuprofen were 15% and 24%, respectively. While at a pH equal to 4.5 the recoveries for both compounds increased until 59 % (naproxen) and 58 % (ibuprofen). This increase in the efficiency is probably due the reducing pH value providing the non-protonated form of the pharmaceuticals drug, increasing its interaction with the stationary phase. However, the phase (C_{18}) with less available silanols is stabilized when lower pH values are used, due to

the fact that it is less susceptible to hydrolysis (Thurman/Mills 1998) as used here. So, the selective retaining and desorption of the pharmaceuticals drugs were significantly improved as has been reported in other research works (Costi *et al.*, 2008; Santos *et al*. 2005).

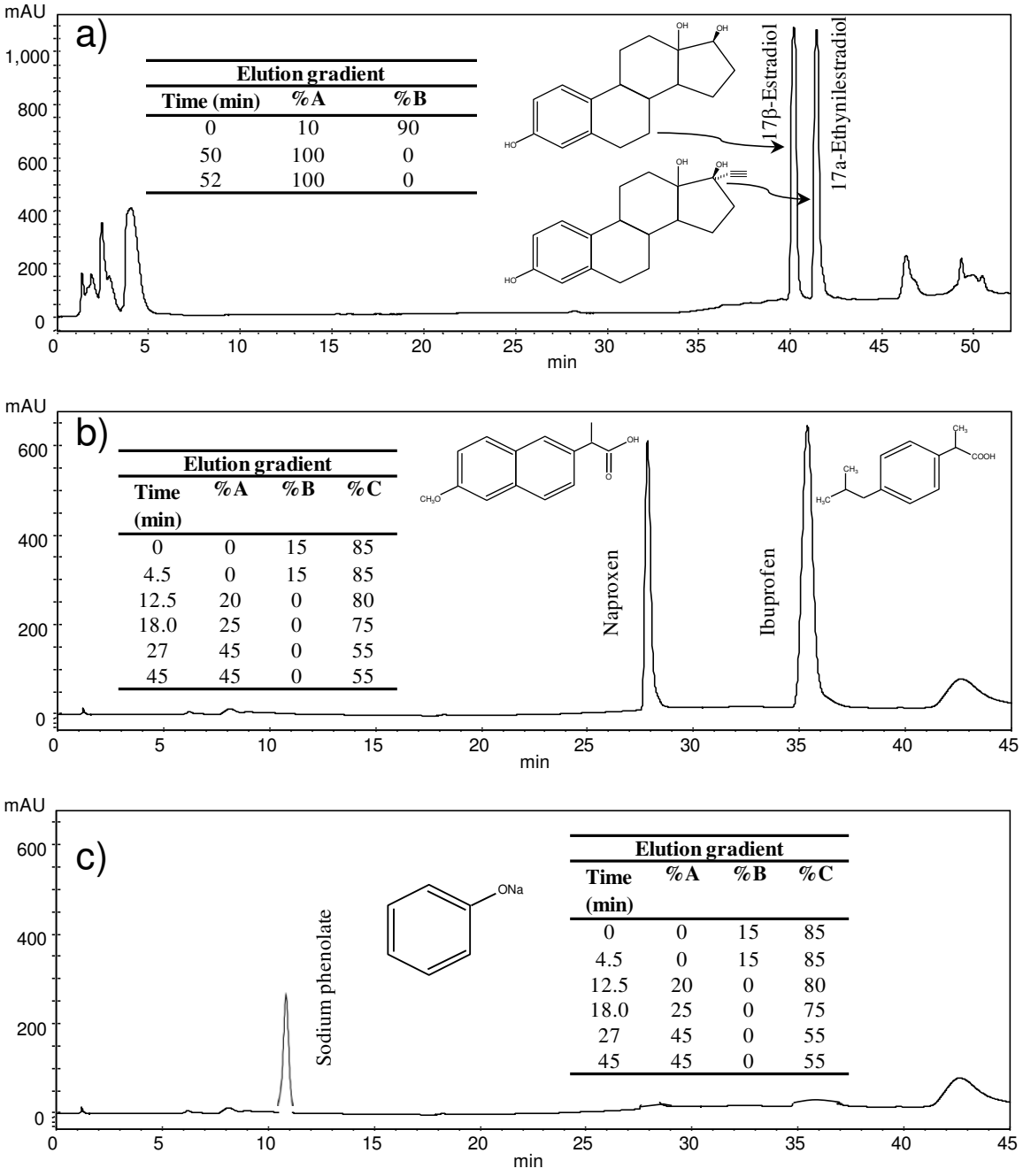

Figure 1. Chromatograms obtained by HPLC analysis of two standard mixtures of a) 50 µg mL^{-1} of E2 (pKa=10.4) and EE2 (pKa=10.5) eluted with A: acetonitrile and B: desionizaded water (18 MΩ) and b) 25 µg mL^{-1} of naproxen (pKa=4.2) and 250 µg mL^{-1} of ibuprofen (pKa=4.9) elute with A: acetonitrile, B: methanol and C: Buffer solution 50 mM KH_2PO_4; chromatogram c was obtained with 100 µg mL^{-1} of sodium phenolate using the same elution gradient of pharmaceuticals.

This modification was considered in carrying out the rest of the experiments and to optimizing the method of extraction by means of SPE for those compounds.

3.3. Selection of Reduction Techniques

The reduction of the eluted extract from the cartridges was carried out in two ways, by means of a gentle stream of chromatographic nitrogen and by using a rotary evaporator to evaluate the effect of the reduction technique over the efficiency of recovery of the analytes. Both techniques were tested with the same amount of the fortification mass (**Table 1**). The recovery was obtained by comparing the response of the mass in the reduced extract with respect to the standard reference. The results showed that the recoveries of the steroids and pharmaceuticals increased when the rotary evaporator was used (**Table 1**). The efficiency of recovery for sodium phenolate (20 μg mL^{-1}) was evaluated only by rotary evaporator, obtaining a 94.6% (CV=5.1).

Table 1. Efficiency of recovery with the two reduction techniques of the extract.

Compounds	% R (CV) with N_2	% R (CV) with Rotary evaporator
[a]E_2	72.6 (2.4)	89.5 (6.5)
[b]EE_2	67.7 (0.1)	80.1 (4.3)
[c]Ibuprofen	23.9 (28.4)	94.0 (2.1)
[d]Naproxen	15.1 (67.3)	93.0 (2.7)

Fortified samples used with [a,b]50 μg mL^{-1}, [c]250 μg mL^{-1} and [d]25 μg mL^{-1},
%R: percentage of recovery, CV: coefficient of variation (*n*=2), N_2: nitrogen.

In the evaporator, the higher recoveries were likely due to the greater control over the conditions of reduction. The lower recoveries, performed by means of the gentle flow of chromatographic nitrogen gas, were likely because a very fast flow was used which produced a significant co-evaporation of compounds and solvents (Jakobsen *et al*. 2003), however further experiments should be performed to demonstrate these results more reliably. Regardless, the reduction using the rotary evaporator presents the advantage of being faster and cheaper than the technique using a gentle flow of nitrogen, because solvent with high vapor pressure, such as acetonitrile and ethyl acetate, require longer times for evaporating, which increases the cost to the use of larger volumes of nitrogen chromatographic gas.

3.4. Assessment of Optimized Conditions

3.4.1. Linearity

The linearity of the proposed spiked range was also evaluated plotting the observed response of absorbance, after the treatment of the sample, against that of the different added mass. The correlation coefficients (r) were greater than 0.993 for all the analyzed compounds ($P<0.05$) (**Table 2**).

3.4.2. Recoveries

The optimized method of extraction in the solid-phase was applied to spiked water samples with different concentration of steroids and pharmaceuticals in order to know the behavior of recovery regarding the amount of analyte present in the sample. Each spiked level was assayed in duplicate and the recovery was calculated by comparing the response in the tested sample with the dissolution of reference standard. First, it was demonstrated that the extraction efficiencies (**Table 2**) were independent of the loaded mass of analyte (ANOVA test $p>0.05$). Therefore, a recovery factor >83% for steroids, more than 93% for pharmaceuticals, and >45.7 % for sodium phenolate, would be applied for adjusting the concentration found in real samples within the evaluated range. The correlation coefficients (r) were superior to 0.993 for all the compounds analyzed and the interceptions of the regression curves were statistically equal to zero ($p < 0.05$) (**Table 2**). Therefore, a recovery factor > 83% for steroids and > 94% for pharmaceuticals would be applied for adjusted the concentration found in real samples within the evaluated range. The recoveries of the steroids are lower than those reported by López de Alda/Barceló, (2000), while for pharmaceuticals, they are in accordance with the average recoveries reported by Santos *et al*. (2005) (naproxen 98% and ibuprofen 89%). Both are satisfactory since the variation introduced by each one of the stages is considered during the treatment of the sample.

3.4.3. Precision

This parameter for quality of the method was represented by the coefficient of variation (CV) and it was evaluated as repeatability for each level of the tested and as reproducibility throughout the levels (n=6 and 8). For steroids the reproducibility obtained was of 3.8 %

(E_2) and 3.3% (EE_2), while for the pharmaceuticals, the values were 5.2 % (ibuprofen) and 3.2 % (naproxen), and 3.1 for sodium phenolate (**Table 2**).

Table 2. Extraction efficiency of steroids and pharmaceuticals at different fortification levels under optimized conditions and lineal regression obtained to estimate the recovery factor.

Steroids			Pharmaceuticals				Competitive compound	
($\mu g\ L^{-1}$)	E_2	EE_2	Ibuprofen		Naproxen		Sodium phenolate	
	%R (CV)	%R (CV)	($\mu g\ L^{-1}$)	%R (CV)	($\mu g\ L^{-1}$)	%R (CV)	($\mu g\ L^{-1}$)	%R (CV)
12.5	82.0(4.1)	90.1(4.6)	**25**	88.0 (2.4)	**2.5**	72.9 (5.2)	**2.0**	37.2 (2.1)
25	89.1(0.8)	91.2(1.0)	**62.5**	95.3(12.1)	**6.25**	102.3(4.5)	**5.3**	30.80 (4.0)
50	89.5(6.5)	80.1(4.3)	**125**	85.0 (3.4)	**12.5**	82.4 (1.0)	**6.7**	43.0 (3.2)
			250	94.0 (2.7)	**25**	93.0 (2.1)		
r	0.999	0.996	r	0.993		0.997	r	0.966
m ± sd	0.969 ± 0.010	0.832 ± 0.032	m ± sd	0.932 ± 0.042	m ± sd	0.938 ± 0.026	m ± sd	0.457 ± 0.053
b ± sd	-54.6 ± 7.7	22.2 ± 18.1	b ± sd	-2.7 ± 4.5	b ± sd	-1.1 ± 1.4	b ± sd	-0.24±0.223

%R: recovery percentage, CV: coefficient of variation (n=2), r: correlation coefficient, m: slope or recovery factor, sd: standard desviation, b:intercept ($\mu g\ L^{-1}$)

3.4.4. Limits of Detection and Quantification of Method

Limits of detection (LOD) and quantification (LOQ) of method were determined in accordance with (Mitra, 2003) and considering each step of samples preparation (extraction, elution, reduction). The LODs for steroids were 1.24 $\mu g\ L^{-1}$ (EE2) and 4.21 $\mu g\ L^{-1}$ (E2) and LOQS were 4.13 $\mu g\ L^{-1}$ (EE2) and 14.04 $\mu g\ L^{-1}$ (E2). Similarly, for pharmaceutical compounds the LODs were 18.6 $\mu g\ L^{-1}$ (naproxen) and 1.48 $\mu g\ L^{-1}$ (ibuprofen) and LOQs were 62.7 $\mu g\ L^{-1}$ (naproxen) 4.93 $\mu g\ L^{-1}$ (ibuprofen), respectively. The LOD and LOQ for sodium phenolate were 1.7 $\mu g\ L^{-1}$ and 5.8 $\mu g\ L^{-1}$, respectively.

3.5. Degradation Efficiency of Steroid

The residual concentration was compared to the initial one and it was used to determine the efficiency of degradation of the E_2. The results (**Table 3**) indicate that the process degrades more than 98% of the steroid, which are similar results to those reported by Maniero *et al*. (2009); Deborde *et al*. (2005) and Huber *et al*. (2003). Also, it was found that the determination of the residual mass (6.72 to 17.8 µg) of the steroid in the treated sample (1L) was favored by the application of the method under optimal conditions, due fundamentally to the fact that the extraction and concentration stages facilitated obtaining the compound at levels that are above the limit of detection method (1.24 mg L^{-1}), contrary

to the case where it would have been directly injected. Besides, the high efficiency of the method of extraction and the correction of the final result by the recovery of the method provide higher reliability for the determination, since in lower recoveries the probability of identifying and quantifying the compound could diminish especially if it is found in lower concentrations and in environmental samples, where there are more interferences.

Table 3. Results of the degradation of steroid E_2 to evaluate the recovery applying the analytical method.

Experiment	[E_2], final (μg L^{-1}) [b,c]	Degradation (%)	CV (%)	Total CV (%)
1	6.72	96.6	12.9	4.7
2	17.84	91.1	1.1	

[a]-LD: 1.3 μg L^{-1}, [E_2] initial = 200 μg L^{-1}, [b]-corrected by recovery factor, 93% and [c]-concentration factor, 10/1000

4. Conclusions

Finally, two efficient and reliable methods were optimized and applied for the determination of two steroids and two pharmaceuticals in water samples by SPE and HPLC-DAD analysis. The recoveries obtained after sample treatment were 83 (EE_2) and 97% (E_2) for the steroids, 93% (ibuprofen) and 94 % (naproxen) for pharmaceuticals and 46% for sodium phenolate.

The estimated detection limits suggested that it is possible to treat similar concentrations to that of environmental samples (μg L^{-1}) with recoveries that do not depend on the amount of compounds in the mass levels assayed. Therefore, the SPE procedure ensures that lower concentrations of steroids and pharmaceuticals are detected, which is important because it facilitates reaching the detection levels provided by the HPLC-DAD. These methods are presented as an accessible alternative simple and economic, since the cost of reactants and materials is lower than those GC-MS techniques and the availability of the chemicals analysis systems is actually possible in most of the research laboratories. Additionally, the applicability of the analytical methods for the determination of residual mass of steroids after a degradation treatment with ozone was demonstrated.

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CHAPTER 3

ASSESSMENT OF THE KINETICS OF OXIDATION OF STEROIDS AND PHARMACEUTICAL COMPOUNDS IN WATER USING OZONE

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Abstract

Emerging Contaminants (ECs) are present in surface and ground waters; however, their elimination by using conventional treatment methods has not been an easy activity. This technical problem generates a potential risk for the public health of surrounding populations that consume this liquid. The aim of this research was the assessment of the kinetic of oxidation of four ECs in water: two steroids, 17β- Estradiol (E2) and 17α-Ethynilestradiol (EE2) and two pharmaceuticals compounds: naproxen (NPX) and ibuprofen (IBP) by using ozonation processes. The stoichiometry and the second order rate constants of the four selected compounds were obtained. The methods of competition kinetics and absolute rate constant under pseudo-first order conditions were established to assess the kinetics of oxidation of steroids, NPX and IBP respectively. The second order rate constant for E2, EE2, NPX were in the order of 10^4 to 10^5 and 10^1 $M^{-1}s^{-1}$for IBP, respectively. The oxidation model obtained for the ECs from the rate constants was represented for different ozone doses and should be compared to the experimental values. In addition, the half-life of the selected ECs for each applied ozone dose was obtained. This research takes part of a project targeted towards water treatment, with presence of ECs at semi-pilot scale under continuous regime.

1. Introduction

The antropogenic activities, particularly industrial ones, produce and use a large range of Emerging Contaminants (ECs), such as pesticides, alkylphenols, dioxins, biphenol A, polycyclic aromatic hydrocarbons, styrene, phtalate, as well as steroids, pharmaceuticals and personal care products (Vallejo-Rodríguez and López-López, 2011; Kümmerer, 2010; USGS, 2008; Snyder *et al*., 2006). The presence of ECs in the environment has raised the interest to research the possible adverse effects in human health and aquatic ecosystems (Carsten *et al*., 2011; EEA, 2010; Kümmerer, 2010), due to the potential danger that some of them have shown in the endocrine system of living beings (Burkhardt-Holm *et al*., 2008; Jukosky *et al*., 2008; Vajda *et al*., 2008).

The wastewaters and effluents from Wastewater Treatment Plants (WWTP) that contains ECs are discharged to the environment, where pharmaceuticals in concentrations of the order of $\mu g\ L^{-1}$ have been detected (Samaras *et al*., 2010; Benotti *et al*., 2009; Radjenović *et al*., 2009; Santos *et al*., 2005); while natural and synthetic steroids are considered the most powerful estrogenic compounds, have been detected in orders of $ng\ L^{-1}$ (Benotti *et al*., 2010, 2009; Jafari *et al*., 2009; Chen *et al*., 2007; Barel-Cohen *et al*., 2006; López de Alda and Barceló, 2000). A feature from the ECs is the recalcitrance of the natural degradation and the conventional processes for water treatment. Therefore, they

remain in the environment for long time (Gomes *et al*., 2010; Peng *et al*., 2008; Suárez *et al*., 2008; Vajda *et al*., 2008). The wastewaters, discharged into the environment with or without treatment, contain ECs at different concentration levels, through the natural infiltrations and runoff. The ECs pollutes surface and ground waters that serve as potable water supplies. This situation puts at risk the population health that consumes the water (Carsten *et al*. 2011; Benotti *et al*. 2010, 2009; Barel-Cohen *et al*. 2006). This environmental and public health issue demands the need to develop treatment processes that are effective towards the degradation of the ECs present in water (Huber *et al*. 2005; Nanaboina *et al*. 2005)**.**

Water treatment with ozone depends mainly of the kinetics of oxidation and the liquid-gas transfer phenomenon (Beltrán, 2004), nonetheless, this research analyzes only the kinetic study without discrediting the importance of the second term. The determination of the rate constants > 1000 $M^{-1}s^{-1}$ can be analyzed with sophisticated equipment such as *stopped-flow and quenched-flow systems.* However, those techniques are expensive and are restricted due to the difficulty of the spectrophotometric analysis of the oxidant (O_3), of the analyte and the sub-products of the oxidation. There are spectral interferences between those chemical species, particularly with aromatic compounds identified in a near wavelength. Facing this analytical issue, the *competition kinetics model* is presented as an alternative to assess the rate constants >1000 $M^{-1}s^{-1}$ and to avoid the above issues (Benítez *et al*. 2008; Deborde *et al*. 2005; Huber *et al*. 2003; Gurol and Nekoulnaini, 1984; Hoigné and Bader, 1983a). The denominated *determination of the absolute rate constant* is used to assess the rate constants that are <1000 $M^{-1}s^{-1}$; it can be performed in two pathways: i) by measuring analyte oxidation and maintaining high and constant ozone concentration; ii) by measuring ozone consumption at different times, maintaining high analyte concentration under pseudofirst kinetic order (Huber *et al.* 2003; Hoigné and Bader, 1983a).

The literature reports several investigations aimed at the study of ECs kinetics. Huber *et al.* (2003) carried out experiments with 4 μM concentrations of E2 and EE2 in Milli-Q water and O_3 concentrations from 1.5 to 7.5 μM at pH=6. The rate constants of second order were in order of 10^6 $M^{-1}s^{-1}$. This study demonstrated the strong dependence between the rate constant and changes in pH. Deborde *et al.* 2005 investigated the oxidation of six ECs, at a pH interval of 2.5 to 10.5 and T=20 °C, including E2 and EE2 with ozone.

The values of the second order rate constants for E2 and EE2 at pH=7 were 2.21 x 10^5 $M^{-1}s^{-1}$ and 1.83 x 10^5 $M^{-1}s^1$, respectively.

Huber *et al*. 2003 carried out the oxidation of 1 μM solution of pharmaceuticals at concentrations of at pH=6 and T=20 °C, with an ozone dose of 1 mM. There was an oxidation of IBP of 55% of the initial concentration at t > 35 min of reaction. The ozonation of the effluents of the WWTP that contained pharmaceuticals and steroids was carried out by Huber *et al*. (2005) at pilot scale with O_3 doses of 10.4 μM to 104 μM. The second order rate constant was ~2 x 10^5 M^{-1} s^{-1} for the NPX assayed. The ozonation of four pharmaceuticals in ultra-pure water including NPX was studied by Benítez *et al*. (2009).

The ozonation of four pharmaceuticals in ultra-pure water including NPX were studied by Benítez *et al*. (2009) at a pH interval of 2.5 to 9. The apparent rate constant of NPX was determined and varied from 2.6 x 10^4 to 3.00 x 10^5 M^{-1} s^{-1} in the assayed interval of pH. Nanaboina *et al.* 2010 carried out the ozonation of wastewaters containing pharmaceuticals and found a second order rate constant of 4.2 x 10^5 and 8.1 x 10^5 M^{-1} s^{-1} for the IBP and NPX, respectively, at pH=7.6 and T=21 °C.

The aim of this research is the assessment of the kinetics of oxidation and half-times of ECs by means of the determination of the stoichiometric coefficient and the second order rate constant, taking two steroids and two pharmaceuticals as model compounds and using ozone at different doses as oxidant. The competition kinetics technique was implemented for the case of the two steroids and NPX. For IBP oxidation the absolute rate constant determination method was used under pseudo-first order conditions. The model compounds were chosen based on their presence in bodies of water and population consumption. E2 was chosen since it is the most powerful steroid in mammals and EE2, the most common estrogen used in combined oral contraceptives. Naproxen (NPX) and Ibuprofen (IBP) were chosen, two non-steroidal analgesic and anti-inflammatory of wide use in medical treatments. The assessment of the kinetics of reaction of the model compounds is a fundamental part of a larger study aimed at the design of chemical reactors to treat polluted waters with ECs.

2. Materials and methods

2.1. Standards and reactives

The standards of the steroids and pharmaceuticals, E2 (98%), NPX (98%), IBP (98%), sodium phenolate (99%), and EE2 (98%) were acquired in powder form from Sigma-Aldrich and Fluka (USA). The reactives used for the solutions (buffers and elution) were reactive grade and were used with no purification. The solvents (HPLC grade), acetonitrile (ACN) and water were acquired from Tedia (USA) and methanol (MeOH) from JT Baker (USA), ethyl acetate was acquired from Burdick & Jackson (USA). The standard solutions of the ECs under study were prepared with deionized Milli-Q water (Millipore).

The concentration of the solutions spiked with steroids and pharmaceuticals was subjected to its solubility in Milli-Q water. Considering this limitation, the solubilities obtained in this study for E2, EE2 at pH=6 and 21 ºC and for IBP and NPX at pH=5 and 21 ºC were 0.5, 3.5, 4 and 14 mg L^{-1}, respectively. The solubilities reported here are similar to those reported by Shareef *et al*. (2006) for E2 and EE2 and by Suárez *et al*. (2008) for NPX and IBP.

The standard solutions of O_3 (0.25 mM) were obtained by saturating demineralized water and by using an *Pacific Ozone G11* ozone generator operating at a generation rate of 18 g h^{-1} and T=20 °C, from oxygen. In IBP oxidation, a solution with larger concentration of O_3 (1 mM) was prepared by means of a thermocirculator and bath at 2 ± 0.5 °C (Huber *et al*. 2003).

2.2. Analytical methods

The selected steroids and pharmaceuticals under oxidation were analyzed by two analytical methods that included Solid Phase Extraction (SPE) to concentrate the ECs. The extracted samples were analyzed by means of High Performance Liquid Chromatography (HPLC) using Varian ProStar 7725 equipment. The detection was carried out with a Varian ProStar 230 diode array detector (DAD) (Walnut Creek, CA); using maximal absorption wavelengths (λ_{max}) of 197 nm for steroids and 220 nm and 230 nm for IBP and NPX respectively. (López de Alda and Barceló, 2000; Santos *et al*., 2005). The SPE-HPLC-DAD showed recovery efficiencies greater than 93% and this factor was applied to quantify the residual concentrations of the model compounds during the ozonation process. More details

about the methods to quantify the steroids and pharmaceuticals can be found in chapter two or Vallejo-Rodríguez *et al*. (2011). Saturation and residual ozone concentration were determined by means of indigo colorimetric method 4500 of APHA, AWWA, WEF (2005).

2.3 Experimental Procedure

2.3.1 Determination of the stoichiometric coefficient

The direct reaction of ozone (O_3) upon a solute M (E2, EE2, NPX e IBP) can be represented by the Equation 1:

$$M + nO_3 \xrightarrow{k_{O_3}} M_{ox} \qquad \text{Eq. (1)}$$

Where *n* is the stoichiometric coefficient that represents the ozone molecule consumed per molecule M transformed to M_{ox} and k_{O_3} is the rate constant of ozone consumption (Hoigné and Bader, 1983a). The parameter *n* is basic to establish the kinetic regime of ozone absorption in fast reactions in water and defines a heterogenic kinetic of reaction of ozone with analyte M to be assessed (Beltrán, 2004).

The determination of *n* was carried out using five volumetric flasks of 100 ml which served as reactors, containing the spiked solution of analyte at constant concentrations and adding different aliquots of a standard ozone solution to maintain an initial molar analyte/ozone rate of ~3:1, for E2 and EE2, and 8:1 for NPX. This ensured a pseudo-first order reaction (Hoigné and Bader, 1983a). The experiments were performed in duplicate and at pH=6 and T=20 ± 1 ºC. The initial concentrations of E2, EE2 and NPX were of 1.75, 6.4 and 20 μM, respectively. After the reaction in aqueous media, between ozone and analyte there is an ozone consumption represented by ($[O_3]_o$-($[O_3]$); there is also an analyte oxidation represented by ($[M]_o$-([M]). The *apparent n value* is defined as the coefficient of consumption of ozone upon analyte oxidation, both in mol, equation (2):

$$n = \frac{([O_3]_0 - [O_3])}{([M]_0 - [M])} \qquad \text{Eq. (2)}$$

When plotting *n* versus different $[M]_0/[O_3]_0$ rates, we obtain a curve whose asymptotic value in the *y* axis represents the *real n value* for high $[M]_0/[O_3]_0$ rates (Beltrán, 2004).

2.3.2 Determination of rate constants

The methodology used to obtain the second order rate constant of the steroids and NPX was competition kinetics, which consists of degrading the analyte of interest along with another competitive compound (reference compound) of the same order of reaction with ozone simultaneously (Hoigné and Bader, 1983a). Obtaining the rate constant of the IBP reaction was done by using the method which denominated absolute rate constant under pseudo-first order conditions, which consists on degrading the analyte of interest with ozone, where the analyte or the oxidant is added in excess. Both methodologies to assess the oxidation kinetics of the ECs were performed at pH=6 and T=20 ± 1 ºC (Hoigné and Bader, 1983a). Terbutyl-alcohol was used in doses of 10-50 mM as scavenger of $OH^{\bullet}$ radical in all the samples (Huber *et al*. 2003).

The competition kinetics consists of the simultaneous oxidation of a reference compound (R) whose second order rate constant is previously known and a target compound whose constant is unknown and which could be obtained directly. The model used by Hoigné and Bader (1983a) is the one that is shown in Equation (3).

$$\ln\left(\frac{[M]_0}{[M]_t}\right)=\frac{n_M}{n_R}\left(\frac{k_{O_3,M}}{k_{O_3,R}}\right)\ln\left(\frac{[R]_0}{[R]_t}\right) \qquad \text{Eq. (3)}$$

Where [M]; n_m and $k_{O_3,M}$ represent the concentration, stoichiometric coefficient and second order rate constant of the target compound, respectively. Also, [R], n_R and, $k_{O_3,R}$ represent the concentration, stoichiometric coefficient and rate constant of the reference compound, respectively. The concentrations of O_3 can be varied to obtain different oxidant doses. For a series of experiments a plot of $\ln[M]_0/[M]_t$ versus $\ln[R]_0/[R]_t$ with a slope of $k_{O_3,M}/k_{O_3,R}$ was made. If $k_{O_3,R}$ is known, $k_{O_3,M}$ can be easily calculated.

Obtaining the kinetic constants of oxidation competitively was carried out with the same analyte and ozone concentrations mentioned in the *n* determination, and at an equimolar rate of analyte with respect to the R. The reference compound used in the E2, EE2 and NPX oxidation was sodium phenolate and its rate constant k_{Fen} was considered constant with value of $2.4x10^5$ $M^{-1}s^{-1}$ and $1.7x10^4$ $M^{-1}s^{-1}$ at pH 6 and 5, respectively (Hoigné and Bader, 1983b).

On the other hand, obtaining the kinetic constant for IBP was carried out by the method which denominated absolute rate constant under pseudo-first order conditions. The direct ozone reaction (molecular way) with the analyte is of second order, Equation (4).

$$-d[M]/dt = k_{O_3}[O_3][M] \qquad \text{Eq. (4)}$$

Where is the second order rate constant of the ozone consumed (Hoigné and Bader, 1983a).

When $[O_3]_0 >> [M_0]$, $[O_3]$ is maintained approximately constant during the time of reaction, it means that the reaction between ozone and analyte are under pseudo-first order conditions. Then, after integration Equation 4, is represented by Equation 5, which refers to a pseudo-first order reaction performed in a batch reactor:

$$\ln\left(\frac{[M]_0}{[M]_t}\right) = k_{obs}\, t \qquad \text{Eq. (5)}$$

$$\text{With} \quad k_{obs} = k_{O_3}[O_3]_0 \qquad \text{Eq. (6)}$$

Where $[M]_0$ and $[M]_t$ are the concentrations of the analyte at zero time and at time t respectively and k_{obs} is the pseudo-first order rate constant which can be determined experimentally by assessing the oxidation of M as a function of time, keeping the conditions specified previously (Huber, 2004).

IBP oxidation was carried out at pH=6 and T=20 °C. A 1000 mL reactor was used with a pipette adapted to the cap of the bottle. The reactor containing a 3.5 μM solution of IBP prepared in deionized water was placed on magnetic stirring equipment. An aliquot of

stock solution of O_3 was added, producing a final rate O_3/IBP of 25:1 (Huber *et al*. 2003; Hoigné and Bader, 1983a). 10 mL aliquots were taken from the reactor by using pipettes every ~5 min, quenching the residual O_3 with 0.1 mL of a 0.5 mM solution of sodium sulfite (Na_2SO_3). The residual O_3 was determined during the first 5 minutes and later every 5 minutes.

3. Results and discussion.

3.1. Stoichiometric coefficients

The stoichiometric coefficients of E2, EE2, NPX, IBP and phenolate reported in the literature are absent. Some of the research carried out by Hoigné and Bader (1983b) report stoichiometric coefficients of 1 for olefin compounds and 2.5 mol O_3/mol for aromatic compounds (including sodium phenolate). The stoichiometric coefficients were considered in our research due to the fact that the kinetic constant of oxidation could be affected according to Equation 3. The molar rate at the beginning of the reaction, between the analyte of interest as well as for the phenolate, were equal to 1, however the molar rate at a time t was ≠1, due to the significant difference in the values of the kinetic constants between the analyte of interest and the phenolate. Therefore, it is expected that the *n* have an influence over the kinetic constants of reaction.

Figure 1 shows the determination of *n* for the assayed analytes. Its value tends to a limit value of 1 when $[M]_o >> [O_3]_o$.

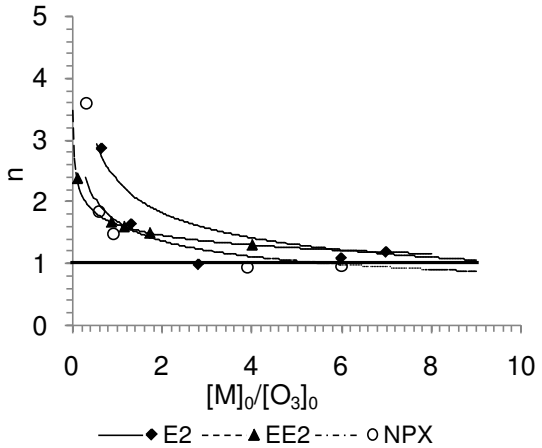

Figure 1. Stoichiometric factor obtained for E2, EE2 and NPX.

Particularly, it could be calculated that the value of *n* is equal to 1.05, 1.15, 0.95 and 0.9 mol of ozone consumed per mol of E2, EE2, NPX and phenolate when $[M]_o/[O_3]_o \geq 6$, which were applied to determine the second order rate constants. For the case of IBP, the value of *n* was not determined, since the determination of its kinetic constant does not require this parameter (Huber, 2004).

3.2. Obtaining the rate constant

To determine the value of the second order rate constant by competition kinetics of E2, EE2 and NPX we first obtained the value of the slope $k_{O_3,M}/k_{O_3,Fen}$ for each compound to be assessed. The value of $k_{O_3,EE2}/k_{O_3,Fen}$ obtained was 0.72 (Figure 2a); from Equation (3) we obtained the value of the constant $k_{O_3,EE2}$ = 0.73 x 10^5 M^{-1} s^{-1} for a pH 6 at 20ºC, with a coefficient of multiple determination (R^2) = 0.99. For E2 and NPX the constant values were 0.9 x 10^6 and 0.10 x 10^5 M^{-1} s^{-1} respectively.

The value of the constant =10.82 M^{-1} s^{-1} for IBP was obtained from Equation 5, whose value of k_{obs} (slope) (Figure 2b) is substituted in Equation 6.

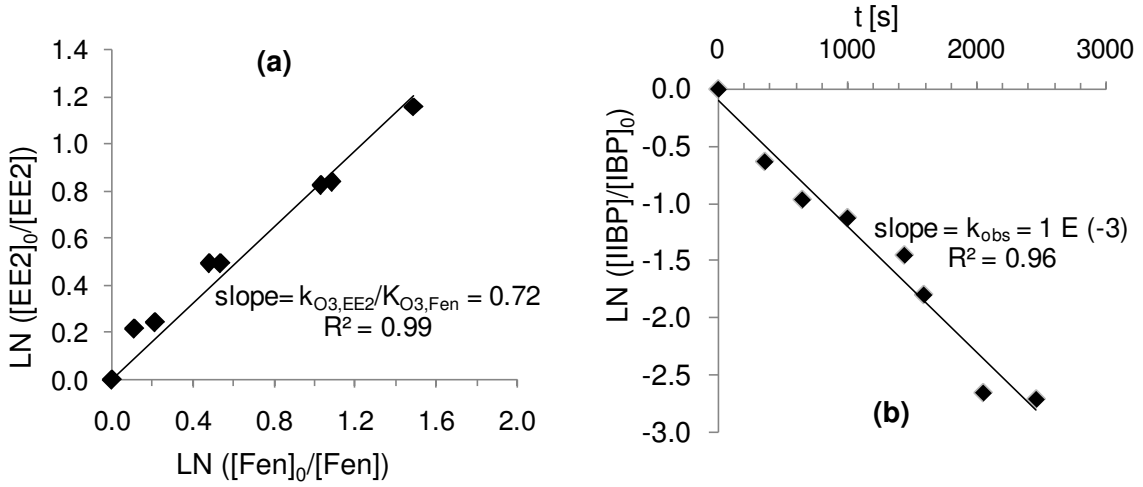

Figure 2.
2(a): Obtaining k_{rel} using competition kinetics
2(b): Obtaining k_{obs} using pseudo-first order kinetics

The application of the competition and pseudo-first order kinetics in this research has yielded similar results to those seen in the literature. The rates of oxidation of second order of the model compounds are summarized in Table 1. The compounds that had rates of

oxidation >1000 M^{-1} s^{-1} were E2, EE2 and NPX, which implies short oxidation times with ozone at the experimental conditions. The IBP had a rate <1000M^{-1} s^{-1} and this is due to the absence of aromatic analytes and reactive groups which are slightly activated similar to those of toluene (Huber *et al*. 2003; Hoigné and Bader, 1983a).

Table 1. Second order Rate constants of the CDE model

Compound	pKa	μM	pH, T (ºC)	Method [M]	Aqueous matrix	Compound of reference	$k_{O_3,M}$ calculated (M^{-1} s^{-1})	Reference
E2	10.4	0.8	6, 20	HPLC-DAD	spiked water	phenolate	0.90 x 10^6	**1)****
		4	6, 20	HPLC	natural water	phenolate	~1.00 x 10^6	**2)****
		1	<5, 20	HPLC	spiked water	phenol	2.21x10^5	**3)****
EE2	10.5-10.7	6.4	6, 20	HPLC-DAD	spiked water	phenolate	0.73 x 10^5	**1)****
		4	6, 20	HPLC	natural water	phenolate	3.16 x 10^5	**2)****
		1	<5, 20	HPLC	spiked water	phenol	1.83 x 10^5	**3)****
NPX	4.2	20	5, 20	HPLC-DAD	spiked water	phenolate	0.10 x 10^5	**1)****
		LD	7.5, 17	EI-NIM	WWTP effluent	--	~2.00 x 10^5	**4)***
		10	5, 20	HPLC-DAD	groundwater and surface water	Penta chlorophenol	0.84 x 10^5	**5)****
		0.043	7.6, 21	HPLC-LC-MS/MS	WWTP effluent	--	8.10x10^5	**6)****
IBP	4.9-5.7	3.6	6, 20	HPLC-DAD	spiked water	--	10.82	**1)***
		1	6, 20	HPLC	natural water	--	9.60	**2)***
		0.048	7.6, 21	HPLC-LC-MS/MS	WWTP effluent	--	4.20x10^5	**6)***

ND: not determined
* Method of apparent constant
** Competition kinetics

LC. DAD: Diode Array Detector
EI: Electrospray ionization
HPLC: High Performance Liquid Chromatography
LC: Liquid Chromatography
LD: Limit of detection
NIM: Negative Ion Mode
WWTP: Wastewater Treatment Plant

1) Research herein
2) Huber *et al*. 2003
3) Deborde *et al*. 2005
4) Huber *et al*. 2005
5) Benítez *et al*. 2009
6) Nanaboina *et al*. 2010

The value of the rate constant of E2 is practically the same as the one obtained by Huber *et al.* (2003). However, the value of this constant is five times larger than the one obtained by Deborde *et al*., 2005. This difference is due to the diverse experimental conditions, specifically pH of the reaction. It has been found that the rate of oxidation of some compounds using ozone could be increased due to the fact that the ionized fraction of the compounds increases with the increase in pH, while the reaction of the neutral fraction

of the compound with ozone could decrease. For example, phenolate is 10 times more reactive with ozone at pH 7 than pH 6 (Deborde *et al.*; 2005; Huber *et al.*, 2003; Hoigné and Bader 1983b).

For EE2, the value of the constant that was obtained in this study is smaller with respect to the one obtained by Huber *et al*. (2003). Nonetheless it is close to the value obtained by Deborde *et al*. (2005). We do not have a solid base which could explain the difference between those compared values.

The value of the rate constant obtained for NPX in the research herein is smaller than the values reported by Nanaboina *et al*. (2010); Huber *et al*. (2005) and Benítez *et al*. (2009). The difference in values is explained by the pH of the reaction and by the competitive compound used. By comparison, the dissociated (anionic) form of naproxen increases with pH and this also allows the increase in the reactivity of ozone due to the acidic nature of the pharmaceuticals. The values of degree of dissociation (α) for NPX at pH 5 and 7 are 0.863 and 0.998, respectively. The alpha values obtained indicate that the k values in the assays were due to a contribution from the dissociated form of naproxen. This is validated by the constants obtained for NPX by Benítez *et al*. (2009) which show values for the direct ozonation of the pharmaceuticals in the neutral and anionic forms of 0.25 x 10^5 and 2.8 x 10^5 M^{-1} s^{-1}, respectively. On the other hand the difference between both could be explained by the type of competitive compound used. For this, research phenolate was used, with a rate constant of 1.7 x 10^4 and Benítez *et al*. (2009) used pentachlorophenol with value of 2.1 x 10^6 M^{-1} s^{-1}. Regarding the difference with the constant obtained by Nanaboina *et al*. (2010), that difference in values is contrasting and it remains explained by the dissociation of the compound in its anionic form.

Regarding the IBP compound, there is a coincidence in the order of the magnitude with the value reported by Huber *et al.* (2003) and the pH used to obtain it. The oxidation of IBP at different pH was carried out by Huber *et al*. (2003). It was found that the second order rate constant is 9.6 $M^{-1}s^{-1}$ for pH 6 to 10. For that, it is considered that the value of the constant obtained in this research ($k_{O_3,IBP}$ = 10.87 $M^{-1}s^{-1}$) for IBP is satisfied. The value reported by Nanaboina *et al*. (2010) for this compound lays in the order of 10^5, however, it is the apparent constant, and the latter includes the oxidation by molecular and radical pathways. Huber *et al*. (2005) have found that the oxidation of IBP could be increased from

40% to 80% with respect to the conventional ozonation using O_3/H_2O_2 for a 10 minute period. The latter implies a change of de pH>7, to favor the radical pathway, which is not explained by Nanaboina *et al*. (2010), since it has been already mentioned that the increase in pH does not have influence in the increase of the value of the constant.

3.3. Oxidation times and half-life times

The second order rate constants were used to represent the oxidation rates for the ECs assessed using equation (3) (continuous lines) and at the same time to compare them to the experimental values (disperse symbols) in Figure 3a, 3b, 3c and 3d.

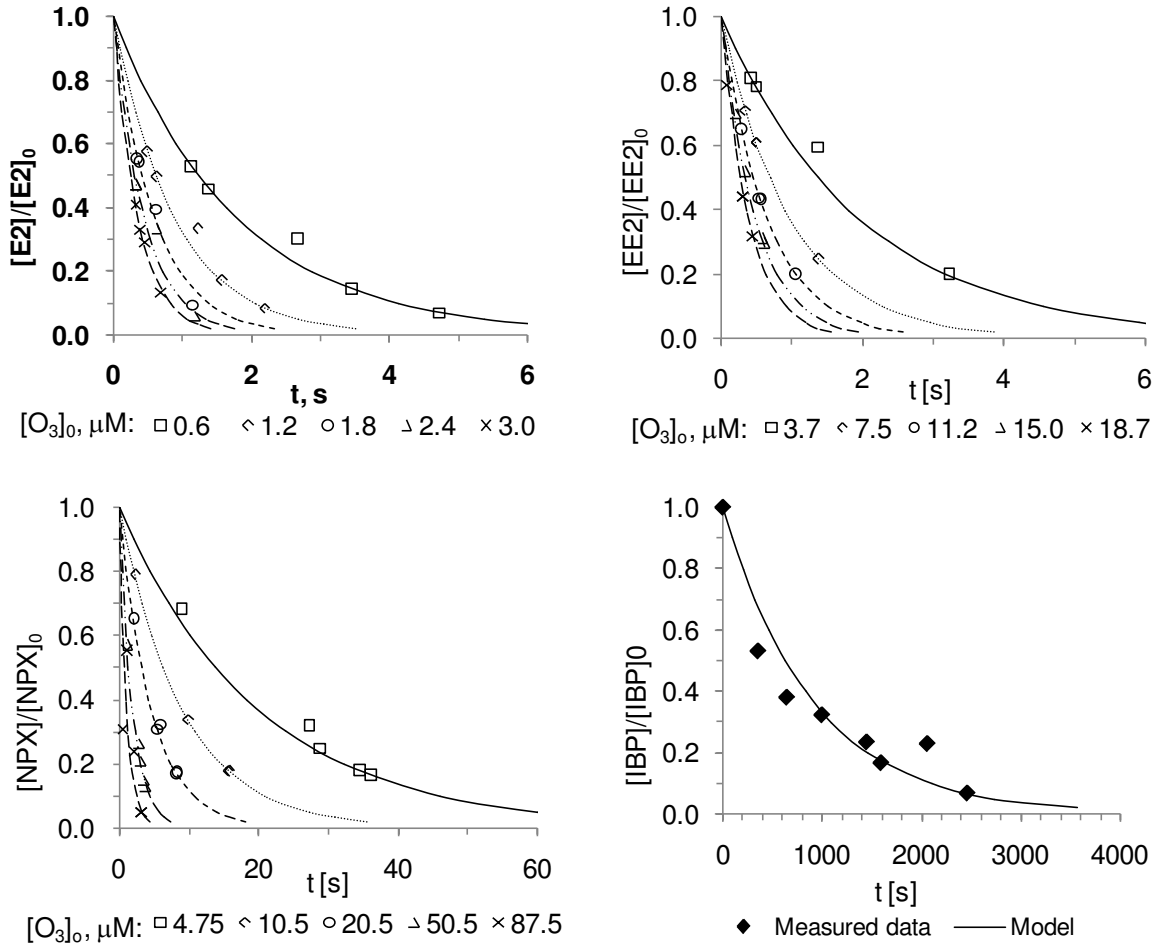

Figure 3. Experimental and modeled oxidation times for E2, EE2, NPX and IBP.

It can be observed that the model (representation of Equation (3)) practically coincides with the experimental points. Additionally, it can be observed that for higher

ozone concentrations, the times required for oxidations of more than 90% of E2 and EE2 are less than 1s, for NPX 3s and for IBP approximately 2000s which confirms that the determination of the kinetic constant of the first three ECs cannot be determined by the absolute kinetics model under pseudo-first order conditions by using the conventional experimental techniques, as per the IBP constant.

The oxidation times of up to 50% of the initial concentration of the ECs assayed (half-life time $t_{1/2}$) were obtained with kinetic parameters previously established as a function of ozone dose. The experimental results are represented in Figure 4. The $t_{1/2}$ in general was less than 1 s for E2 and EE2 for all O_3 dose assayed, excepting for NPX which is < 5 s for the smallest dose of O_3. The $t_{1/2}$ found in the research herein were 0.27 s ($[O_3]$ = 19 μM) and 633 s ($[O_3]$ = 100 μM) for EE2 and IBP respectively. The $t_{1/2}$ found and reported here are similar to those reported by Huber *et al*. (2003). The logarithmic trends models applied to the data of the three compounds show R^2>0.90 indicating that the adjustment to the second order kinetics oxidation model is a function of the ozone concentration.

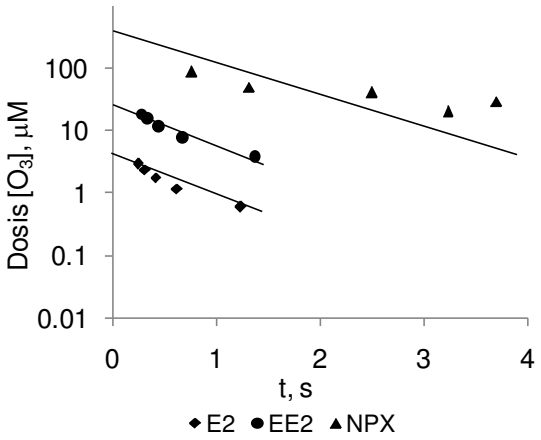

Figure 4. Half-life times of E2, EE2 and NPX for the assayed ozone doses in competition kinetics

4. Conclusions

This research assessed the kinetics of oxidation of four ECs (E2, EE2, NPX and IBP) using ozone. The stoichiometric coefficients *n* of the main reaction indicate that ~1 mol of O_3 is necessary to oxidize 1 mol of E2, EE2 and NPX. The oxidation rates of the steroids and naproxen with ozone are fast; it takes a very short times, <1s to oxidate those compounds;

the method of competition kinetics resulted as the most adequate one to determine the second order rate constant k_{O_3} >1000 M^{-1} s^{-1}; obtaining constant values $k_{O_3,M}$ of the order of 10^4-10^5 M^{-1} s^{-1} for E2, EE2 and NPX. In contrast, the rate of reaction of the IBP with ozone is slow, it requires longer periods of >2000s to degrade this compound, the value of the rate constant is of the order of 10^1 M^{-1} s^{-1}, the method of absolute constant rate resulted as the most adequate one to determine its value. By using the constants $k_{O_3,M}$ a model of the kinetics of oxidation of the ECs was established, which helped in the validation of the experimental data. The methods to determine the second order constants used in this research are robust, reliable and affordable, since they do demand neither sophisticated equipment nor high costs. Finally, the establishment of a method to calculate $t_{1/2}$ of the ECs was attained, as a function of the doses of O_3 applied, which would be useful to predict the models of oxidation of other compounds and in the design of reactors to treat water with ECs.

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GENERAL CONCLUSIONS

The development of this dissertation has met the objectives set at the beginning of the research, the following conclusions were reached. They allow the continuation of this research with the next stage aimed at working with complex aqueous matrices and real conditions.

1. It established the state of the art about the analytical methods and treatment of ECs in water. The literature review conducted reports that a large number of water bodies are contaminated with the ECs and EDCs. Most analytical methods implemented relate to the type of liquid chromatography or gas, either with Diode Array Detector or mass spectrometry coupled with tandem arrangement. Treatment of samples is very important, due to the occurrence of the samples in amounts of ng L^{-1} and μg L^{-1}, so the Solid Phase Extraction is widely used in the management of environmental samples. The conventional treatment processes achieve low efficiencies of degradation of ECs and EDCs, so that the application of Advanced Oxidation Processes (using ozone) treatment is necessary and feasible in solving the problems of treatment.
2. It established the optimized analytical methods used for the quantification of pharmaceuticals and steroids. The recoveries of the compounds were above 80%, with low coefficients of variation CV<5%. The recoveries were independent of the aggregate mass of analyte with repeatability less than 6.5% and 12.1% in steroids and pharmaceutical drugs. Analytical methods were reliable, so recovery percentages assured their use for quantification of the compounds.
3. Competitive kinetics for the oxidation of estradiol, ethinylestradiol and naproxen, and absolute kinetics for ibuprofen, were determined by suitable methodologies. This work established competitive kinetics less than 5 seconds. Particularly, the method of competition kinetic was possible without using costly techniques as the s*topped flow or quenched flow.* If it has these technologies, it is possible to obtain real-time oxidation of the test compound, and determining the reaction products produced through a mass spectrometer coupled to stopflow equipment.

4. Oxidation kinetics constant of ECs using ozone are similar to results reported by other authors. The reaction rate constants are in the order of 10^4-10^5 $M^{-1}s^{-1}$ for steroids, 10^4 $M^{-1}s^{-1}$ for naproxen and 10^1 $M^{-1}s^{-1}$ for ibuprofen.

   In this work, kinetics constants determined by competitive kinetics were obtained using experimental stoichiometric coefficients although reported values were determined without using stoichiometric coefficients.
5. Experiments made in batch reactors indicate that higher doses of oxidant generated reaction times less than 1 s; reaching 90% efficiency of analyte oxidation.
6. A mathematical model was established to represent the kinetics of the ECs oxidation using ozone. The model predicts the time of oxidation for different doses of ozone.
7. Oxidation kinetics of selected compounds will allow us to establish certain studies in heterogeneous media, such as wastewater.
8. Finally, this research contributes to the environmental scientific field, which will allow in the near future developing advanced oxidation process to solve the technical problem on the treatment of complex matrices aqueous contaminated with ECs.