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Abstract

Emerging pollutants (EPs) are a new class of important organic pollutants, most notable for being rarely studied or investigated, whilst believed to have potentially disturbing eco-toxicological character, and most concerning even, for not yet being regulated or controlled by water-quality legislation. They are divided in several sub-classes, amongst which personal-care products and pharmaceuticals are two of the most important.

Ultraviolet (UV) filters are mostly synthetic organic compounds that represent the primary components of sunscreens and related products or pharmaceuticals. They absorb UVB (290-320 nm) and/or UVA (320-400 nm) radiation and therefore provide protection against its effects on human skin and health. Their role and significance implies compelling stability, however, they are known to undergo degradation, photo-induced upon exposure to UV-radiation, and disinfection-induced when in contact with disinfecting agents such as chlorine in swimming-pool waters. On the other hand, pharmaceuticals, despite being similar to UV filters, are aimed at diagnosis and treatment of diseases and all kinds of health afflictions.

The present dissertation is focused on providing a compelling insight on the transformation reactions of several UV filters and pharmaceuticals. In specific, the studies carried out will, more prominently, address the degradation either induced by active chlorine or artificial photo-radiation, the determination of transformation by-products of both processes, physical-chemical properties and environmental persistence. Therefore, this line of investigation will enable a compelling conclusion as to the compounds' stability and environmental impact in the natural aqueous compartments.

Keywords

Ultraviolet filters; UV filters; emerging pollutants; EPs; pharmaceuticals; disinfection-induced degradation; active chlorine; photo-induced degradation; disinfection by-products; DBPs.

Resumo

Poluentes emergentes (EPs) são uma nova e prioritária classe de poluentes orgânicos, conhecidos por não serem alvo de habitual estudo, apesar de estarem potencialmente associados a potenciais implicações eco-toxicológicas preocupantes e não estarem controlados por legislação de controlo de qualidade de água. Esta classe de compostos está dividida em diversas sub-classes, das quais os produtos de higiene pessoal e fármacos são talvez as mais importantes.

Filtros ultravioleta (UV) são, essencialmente, compostos orgânicos sintéticos que representam os elementos fundamentais de protectores solares e outros produtos comerciais ou farmacêuticos relacionados. Estes compostos absorvem radiação UVB (290-320 nm) e/ou UVA (320-400 nm), protegendo assim a pele e a saúde humanas dos seus efeitos nocivos. O seu papel pressupõe assim uma estabilidade significativa, todavia, estes compostos são conhecidos por experimentarem degradação, foto-induzida, após exposição à radiação solar, ou induzida por produtos desinfectantes, quando em contacto com agentes desinfectantes como o cloro em águas de piscinas. Os fármacos, por outro lado, apesar de estruturalmente similares aos filtros, têm como propósito o diagnóstico e combate de doenças e todos os tipos de aflições de saúde.

A presente dissertação está direccionada em providenciar uma perspectiva minuciosa relativamente às reacções de transformação de vários filtros UV e fármacos. Estas reacções são fundamentais, uma vez que os sub-produtos de transformação apresentam habitualmente perfis eco-toxicológicos potencialmente nocivos. Em particular, os estudos elaborados irão essencialmente concentrar-se na abordagem dos processos de degradação, induzidos por cloro ou foto-irradiação artificial, determinação dos correspondentes sub-produtos de transformação, propriedades físico-químicas e características de persistência ambiental. Em suma, esta linha de investigação apresentará considerações conclusivas acerca da estabilidade destes compostos e o seu impacto ambiental em águas superficiais naturais.

Palavras-chave

Filtros ultravioleta; filtros UV; poluentes emergentes; EPs; fármacos; degradação induzida por desinfecção; cloro activo; foto-degradação; sub-produtos de desinfecção; DBPs.

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Acronyms

3BC (3-Benzylidene camphor);

α -HCH (alpha-hexachlorocyclohexane)

BCF (bio-concentration factor);

BCS (benzylidene camphor sulfonic acid);

BDM (4-*tert*-butyl-4-methoxydibenzoyl methane);

BNMs (bromonitromethanes);

BP2 (benzophenone-2);

BP3 (benzophenone-3);

BP4 (benzophenone-4);

BP8 (benzophenone-8);

CBM (3-benzylidene camphor);

CTD (characteristic transport distance);

DBPs (disinfection by-products);

DBT (diethylhexyl butamido triazone);

DHH (diethylamino hydroxybenzoyl hexyl benzoate);

DOM (dissolved organic matter);

DPD (disodium phenyl dibenzimidazole tetrasulfonate);

DRT (drometrizole trisiloxane);

EDP (ethylhexyl dimethyl PAB);

EMC (ethylhexyl methoxycinnamate);

EMT (bis-ethylhexyloxyphenol methoxyphenyl triazine);

EPs (emerging pollutants);

ES (ethylhexyl salicylate);

ET (ethylhexyl triazone);

EU (European Union);

HS (Homosalate);

IAs (iodo acids);

IMC (isopentyl *p*-methoxycinnamate);

K_{oc} (organic carbon distribution coefficient);

K_{ow} (octanol-water partition coefficient);

K_{oa} (octanol-air partition coefficient);

K_{aw} (air-water partition coefficient);

LRTP (long-range transport potential);

MBC (4-methylbenzylidene camphor);

MBT (methylene bis-benzotriazolyl tetramethylbutylphenol);

MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, known as mutagen-x];

OCR (octocrylene);

P15 (polisilicon 15);

P25 (PEG-25 PAB);

PAB (*p*-Aminobenzoic acid);

PBC (polyacrylamidomethyl benzylidene camphor);

PBS (phenyl benzimidazole sulfonic acid);

POPs (priority organic pollutants);

P_{ov} (overall persistence);

STE (superficial transport efficiency);

TDS (terephthalidene dicamphor sulfonic acid);

THMs (tri-halogenated-methanes);

UV (ultraviolet radiation);

UVA (ultraviolet-A radiation);

UVB (ultraviolet-B radiation);

UVC (ultraviolet-C radiation);

Scientific work published or presented in the scope of this dissertation

Santos, A.J.M., Esteves da Silva, J.C.G. (2015), UV filters, their degradation reactions and eco-toxicological effects, in: *Sunscreens – Properties, Role in Skin-cancer Prevention and Health Effects*, Sharp, S.H. (Editor), Nova Science Publishers, NY, USA.

Santos, A.J.M., Esteves da Silva, J.C.G. (2017), Fate and behaviour of the UV filter 3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate (IMC) in aqueous solution, *Journal of Environmental Chemical Engineering* 5 (3), 2469-2479.

Santos, A.J.M., Esteves da Silva, J.C.G. (2018), Environmental fate and behaviour of benzophenone-8 in aqueous solution, article accepted for publication in *Environmental Technology and Innovation*.

Santos, A.J.M., Esteves da Silva, J.C.G. (2018), Fate and behaviour of the UV filter and emerging pollutant hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHBB) in aqueous solution, article submitted, currently in review and awaiting publication.

Santos, A.J.M., Esteves da Silva, J.C.G. (2018), Fate and behaviour of prominent pharmaceuticals, paracetamol, 17 α -ethynylestradiol and carbamazepine, in aqueous solution, article submitted, currently in review and awaiting publication.

Santos, A.J.M., Miranda, M.S., Esteves da Silva, J.C.G., QSAR study of UV filters and their chlorine-induced degradation reactions, *Society of Environmental Toxicology and Chemistry (SETAC) Europe 25th Annual Meeting*, Barcelona, Spain, 3-7 May, 2015.

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Santos, A.J.M., Esteves da Silva, J.C.G., Fate and behaviour of (2-hydroxy-4-methoxyphenyl)-(2-hydroxyphenyl)-methanone (benzophenone-8) in aqueous solution, 25th National Meeting of the Sociedade Portuguesa de Química (SPQ), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal, 16-19 July, 2017.

Chapter 1 – State of the art

1. Introduction

1.1. Emerging pollutants

The fate and behaviour of organic pollutants, as well as their eco-toxicological profiles, is currently one of the most prominent topics in environmentally-oriented research, in particular, regarding the superficial, aquatic, environmental compartments. In the context of these organic pollutants, a new and significant class has recently emerged, being named precisely by emerging pollutants (EPs).

EPs have been generally designated as compounds seldom studied or approached by environmental research, that are believed to display serious and deliterious ecotoxicological profiles and are not yet bound by water-quality regulations [1]. The most iconic subclasses of EPs are presented in [Figure 1](#) of the present Section, of which personal-care products (PCPs) and general pharmaceutical products must be highlighted for their distinctive relevance as part of the group of most prevalent aquatic, synthetic, organic pollutants. Given their similar origins, these two sub-classes of EPs are often grouped together by using the same acronym: pharmaceuticals and personal-care products (PPCPs).

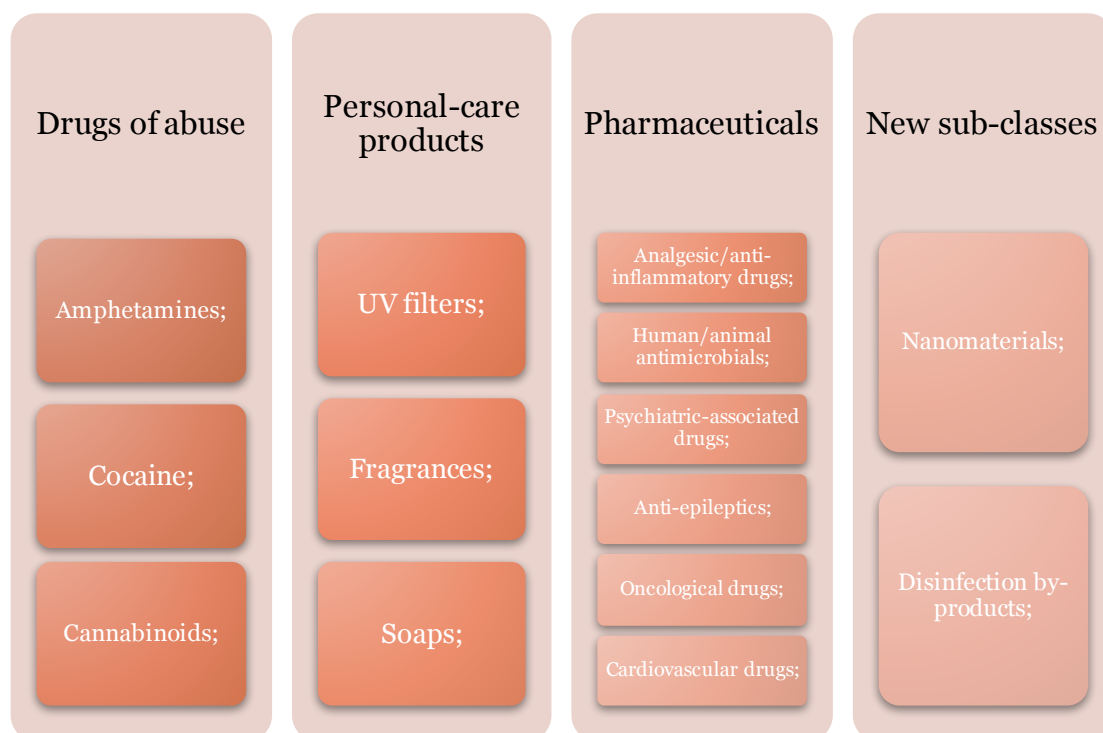


Figure 1. Most important sub-classes of emerging pollutants. Adapted from and inspired by [1].

The inclusion of EPs into the superficial aquatic environments, will be a subject to cover more extensively in the forthcoming sub-sections, but since we are approaching compounds of inherent human/animal applications, the uses given to these components are of crucial importance and will therefore affect their fate and behaviour [1, 2]. After entering the superficial water courses, these compounds will consequently be exposed to biological, chemical and photochemical degradation processes, which actually represent a natural control system to eliminate pollutants. However, an additional problem is presented by the fact that these processes might actually promote the formation of more problematic compounds, in the sense of their increased persistence and more troublesome ecotoxicological profiles [1, 3-5].

The focus of this dissertation, will be aimed at two of the sub-classes of EPs presented in Figure 1, namely prominent pharmaceuticals and personal-care products (more specifically UV filters, the primary constituents of sunscreens). The two sub-classes will be addressed in more detail in the following sub-sections.

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1.1.1. UV filters

The present section was written and published in the scientific book “Sunscreens: Properties, Role in Skin Cancer Prevention and Health Effects”, in response to an invitation from Nova Science Publishers, Incorporated, to our research group led by Professor Dr. Joaquim Carlos Gomes Esteves da Silva.

The structure and content of the book chapter was defined by the authors in accordance to the suggestions made by the aforementioned scientific publisher, as far as the topic to cover and discuss. The text was written by the author, Albano Joel Moreira Santos, whilst guidance, supervision and revision was ensured and provided by Professor Dr. Joaquim Carlos Gomes Esteves da Silva.

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Chapter 5

UV FILTERS, THEIR DEGRADATION REACTIONS AND ECO-TOXICOLOGICAL EFFECTS

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ABSTRACT

Sunscreens or sunscreen agents are more notoriously known as ultraviolet (UV) filters, and they are the prime components of many personal care products and pharmaceuticals. Most UV filters are organic compounds that absorb UV radiation, therefore protecting us from solar radiation and its nefarious effects on human skin and health. The protective character of UV filters regarding UV radiation, would presuppose a stable nature towards alterations in general. However, the compounds are well known to undergo degradation, and in many cases quite substantially, either by influence of UV radiation itself (by photolysis or photo-isomerization) or through contact with water disinfecting agents, such as chlorine. These degradation reactions might be quite troublesome, since they generate degradation by-products that either do not present the appropriate UV-protective capabilities, as is the case with photo-isomers, or possess toxicological profiles potentially damaging for both the human

health and the environment, as is the case with free-radicals or even disinfection by-products (DBPs).

INTRODUCTION

Ultraviolet (UV) radiation constitutes about 6.2% of the total solar radiation that is able to reach the Earth's surface, given the filtration and mitigation capabilities of the protective ozone layer. Out of this specific portion of the solar radiation, mostly is attributed to UVA radiation (320-400 nm) while a very small portion is attributed to UVB radiation (290-320 nm).

Table 1. Physical-chemical properties of the UV filters currently approved and regulated in and by the EU (adapted from [3] and [6]).

Structure	INCI name	Acronym	Molecular weight	Log K _{OW} ^d	Log BCF ^{e, h}	Log K _{OC} ^{f, h}	Solubility /g/L ^g	λ _{max} /nm
Benzophenones	Benzophenone-3	BZ3	228.24	3.79	1.38	3.10	0.21	290
	Benzophenone-4	BZ4	308.31	0.88	-	-	0.65	240 ^b ; 288
PABA and derivatives	<i>p</i> -Aminobenzoic acid	PAB	137.14	0.83	-	-	915	282
	PEG-25 PABA	P25	277.41	-	-	-	-	310 ^a
	Ethylhexyl dimethyl PABA	EDP	277.40	6.15	3.74	3.38	2.1×10 ⁻³	310
Salicylates	Homosalate	HS	262.35	6.16	-	-	0.02	-
	Ethylhexyl salicylate	ES	250.34	5.77	-	-	0.028	240 ^a
Cinnamates	Ethylhexyl methoxycinnamate	EMC	290.40	5.80	5.80	4.10	0.15	306 ^b
	Isopentyl <i>p</i> -methoxycinnamate	IMC	248.32	4.06	-	-	0.06	-
Camphor derivatives	Camphor benzalkonium methosulfate	CBM	409.55	0.28	-	-	-	288 ^a
	Terephthalidene dicamphor sulfonic acid	TDS	562.69	1.35	-	-	0.014	340 ^a
	Benzylidene camphor sulfonic acid	BCS	320.40	2.74	-	-	0.038	297 ^a
	Polyacrylamidomethyl benzylidene camphor ^d	PBC	-	-	-	-	-	-
	4-Methylbenzylidene camphor	MBC	254.37	4.95	3.51	3.89	5.1×10 ⁻³	300 ^b
	3-Benzylidene camphor	3BC	240.34	4.49	-	-	9.9×10 ⁻³	292 ^a
Triazines	Ethylhexyl triazone	ET	826.10	15.53	-	-	-	310 ^a
	Diethylhexyl butamido triazone	DBT	765.98	11.90	-	-	4.6×10 ⁻⁷	-
	Bis-Ethylhexyloxyphenol methoxyphenyl triazine	EMT	627.81	13.89	-	-	4.9×10 ⁻⁸	340 ^b
Benzotriazoles	Drometrizole trisiloxane	DRT	225.25	9.79	-	-	1.3×10 ⁻⁵	344; 303
	Methylene bis-benzotriazolyl tetramethylbutylphenol	MBT	658.87	14.35	-	-	3.0×10 ⁻⁸	340 ^b
Benzimidazole derivatives	Phenyl benzimidazole sulfonic acid	PBS	274.30	0.01	0.50	2.46	0.26	300 ^b
	Disodium phenyl dibenzimidazole tetrasulfonate	DPD	674.60	-	-	-	-	250
Dibenzoylmethane derivatives	Butyl methoxydibenzoyl methane	BDM	310.39	2.41	4.51	3.23	0.037	358 ^a
	Diethylamino hydroxybenzoyl hexyl benzoate	DHH	397.51	6.93	-	-	9.5×10 ⁻⁴	360 ^b
Others	Octocrylene	OCR	361.49	7.35	-	-	2.0×10 ⁻⁴	300 ^b
	Polysilicon 15	P15	-	-	-	-	-	313 ^c

Data is originated from SciFinder, American Chemical Society, 2008.

The UV filters shadowed in green colour, represent the most popular and frequently used compounds in commercial formulations of sunscreen products.

^a Rastogi, S.C., Jensen, G.H. (1998), Identification of UV filters in sunscreen products by high-performance liquid chromatography–diode-array detection, *J. Chromatogr. A* 828 (1-2), 311-316.

^b De Orsi, D., Giannini, G., Gagliardi, L., Porrà, R., Berri, S., Bolasco, A., Carpani, I., Tonelli, D. (2006), Sample extraction and HPLC determination of UV-A and UV-B filters in sunscreen products, *Chromatographia* 64 (9-10), 509-515.

^c Philippe Maillan Formulation, R&D Cosmetics, DSM Nutritional Products; Measurement of UV Protection in Hair.

^d Octanol-water partition coefficient (K_{OW}); it regards the ratio between the concentration of a substance in octanol and in water, in equilibrium and at a determined temperature.

^e Bio-concentration factor (BCF); it regards the concentration of a substance in an organism and in the water body around it.

^f Organic carbon distribution coefficient (K_{OC}); it regards the ratio between the mass of a substance adsorbed into the soil (by unity of mass of organic carbon in the soil) and the concentration of the same substance in equilibrium in solution.

^g In water and at 25°C.

^h Giokas, D.L., Salvador, A., Chisvert, A. (2007), UV filters: from sunscreens to the human body and the environment, *Trends in Analytical Chemistry* 26 (5), 360-374.

The highly energetic UVC radiation (100-290 nm) is completely blocked by the ozone layer and therefore does not reach the surface of the planet [1]. Despite the beneficial character of UV radiation (it enhances the production of vitamin D, improving the human resistance towards different pathologies; increases the calcium absorption by the organism; etc.), it is also known to enhance the occurrence of skin cancer, as well as other serious but less prominent issues like inflammations, sunburns, and allergic reactions [1]. It is in this context that sunscreens or sunscreen agents play a fundamental role, by preventing or attenuating the damaging effects of UV radiation on the human skin and health [1].

Sunscreen products include complex formulations of different compounds, of which UV filters are of the utmost importance, since these are the compounds that indeed protect us from UV radiation. As Salvador and Chisvert [2] refer, a sunscreen is defined as any product containing UV filters in its formulation, in order to protect the skin from the negative effects of UV radiation, significantly decreasing its impact on human health [2]. The mechanism of protection, however, is based on two processes that are intimately linked to the two existing types of filters in question: essentially absorption of UV radiation in the case of the vastly more numerous organic UV filters; and reflection or scattering in the case of the few existing inorganic UV filters [3].

UV filters generally display either simple or multiple aromatic structures, often conjugated with carbon-carbon double bonds or carbonyl groups, which attributes them the ability to absorb or scatter UV radiation. These compounds will absorb UV radiation, therefore evolving towards a superior energetic state but returning thereafter to the original state by emitting energy through vibrational transitions or photochemical reactions [4]. As it was already mentioned, UV filters are classified as either organic (UV-absorbent) or inorganic (UV-scattering) compounds. The most prominent classes of UV filters are the benzophenones, salicylates, cinnamates, triazines, *p*-aminobenzoic acid derivatives, dibenzoyl methane derivatives and camphor derivatives, and there are globally about 55 filters approved, regulated and controlled worldwide, out of which merely two are inorganic (zinc oxide and titanium dioxide) [1, 4, 5].

Table 1 includes all the UV filters currently approved in the EU, as well as all their relevant physical-chemical properties [3, 6].

As for the nature of UV filters, these compounds present the features common to

most priority organic pollutants (POPs), such as the presence of aromatic rings in association with long and unsaturated aliphatic chains. Most of the filters consist of geometrical isomers (*E* and *Z* forms), although the commercial formulations include solely the *E* isomer. As is visible in Table 1, these compounds exhibit commonly increased lipophilicity, enabling their association with particles rich in organic matter content, such as soils and sediments, as well as high resistance towards biotic degradation, which enhances their accumulation, concentration and persistence in the environment and the food chain [3, 6, 7].

UV Filter Degradation Reactions

Regarding their purpose of application, protection of human skin from the effects of UV radiation, there is the assumption that UV filters are quite stable to general degradation.

However, such assumptions are not exactly accurate, in fact, it is well reported and established that UV filters experience degradation from two essential sources: photo-degradation, upon exposure to UV radiation; and degradation induced by disinfecting agents such as chlorine, when in contact with these in aqueous solution [3, 4]. Figure 1 presents the paths of degradation and their consequential by-products.

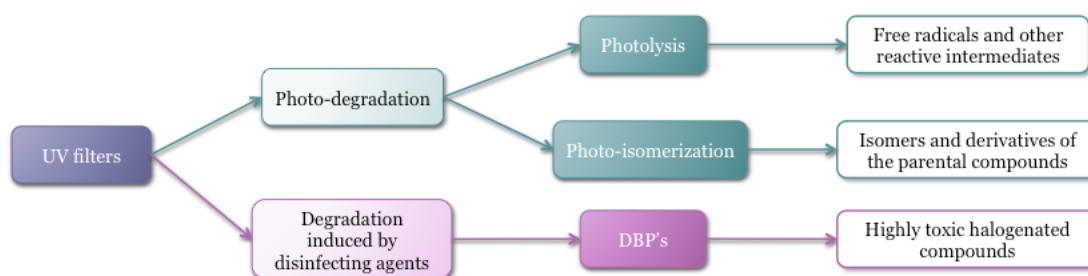


Figure 1. Degradation processes experienced by UV filters.

Photo-degradation of UV Filters

Photolysis

The direct dissociation of a molecule upon the absorption of a determined amount of energy from a given type of radiation is ever more likely when that amount is equivalent or higher than the bonding energy of that same molecule. When this

molecule reaches a higher or excited energy state, it dissociates, and the process is regarded as photolysis [14].

Photolysis is usually a rather complex set of reactions that lead to the formation of reactive species or fragments, and it can be experienced either by direct or indirect paths. Direct photolysis occurs upon the absorption of radiation by specific portions of the UV filters' structure itself, denominated chromophores. Indirect photolysis occurs upon the absorption of radiation by other structures or compounds rather than the UV filter, named photosensitizers, therefore initiating a series of reactions that will induce the transformation or degradation of the filters [3, 8, 9, 14]. Organic compounds will also experience degradation when in contact with reactive species, such as singlet oxygen, hydroxyl radicals, photo-excited organic matter and others [8, 9].

This type of photochemical reactions is one of the most important abiotic processes that control the fate and behaviour of UV filters when in the environment, in particular the aquatic compartments, and in general their prominence is far more significant than the biotic processes of degradation [3, 8], which will be approached in more detail further ahead.

There are numerous studies that have dealt with the subject of photo-degradation, with emphasis on photolysis. The general notion that must be underlined is that as the filters are exposed to UV radiation, they gradually lose their UV-protective features or capabilities [10, 11], which is also accompanied by the formation of several toxic and harmful by-products, as it has been demonstrated with EMC [12]. In his work, Sayre [13] stressed the complexity of the photo-degradation issue, since UV filters are used as part of a formulation of several different filters in commercial sunscreen products, and not in singular. In other words, the photochemical profile and behaviour of a matrix of multiple filters is fundamentally different than that displayed by a single UV filter, since the photo-degradation reactions of many filters are known to enhance or even induce the degradation reactions of others, even those supposedly more stable [13]. Serpone has also carried out some interesting studies on the photo-degradation or photo-stability of certain UV filters [10, 11]. His approach [11], delved on the photo-degradation of different UV filters and its extension in aerobic aqueous medium. The study was carried on the basis of the record of any and every alteration to the UV radiation absorbance as a function of irradiation time, with any loss of absorbance being subsequently directly correlated with loss of UV-

protective capabilities. Results have shown that, in the case of the filter PAB, for instance, UV radiation absorbance capacity decreased about 35% just within the first hour of irradiation, whereas in the case of a very similar filter, EDP, the UV absorbance decrease was almost complete just after 20 minutes of irradiation. As for TDS, it was defined as the most photo-unstable UV filter of the study, with 90% UV radiation absorbance decrease after just 10 minutes of irradiation, while BZ3 was considered the most photo-stable, with a UV absorbance decrease of 20% and throughout two hours of irradiation. Photo-degradation studies have always focused on its relation towards photo-protection alterations [10], ability of the filter mixture to enhance photolysis [10, 13], or the toxicological potential of by-products [12], but seldom has it focused on degradation in the environmental context [15]. Sakkas [15] approached simultaneously the disinfection by-product (DBP) formation as well as the photo-degradation by-products. Results have shown that photochemical reaction rates depend, not only on the environmental conditions, but also on the presence of other relevant compounds in solution, in particular dissolved organic matter (DOM). In the case of the filter EDP, degradation decreased significantly with the increase of DOM levels in solution, which is easily explained by the fact that DOM actively competes with any other present organic compound for the incident photons, in regards to photo-degradation. The authors were also able to identify several photo-degradation by-products, namely from dealkylation and hydroxylation, and in all the different sources of water samples studied (distilled water; swimming-pool water; and sea water).

Photo-Isomerization

Contrary to photolysis, photo-isomerization reactions yield new species closely related to the parental structures, but potentially more toxic and harmful than the original compounds. Regarding UV filters in particular, this translates essentially into the production of photo-isomers that may be related but no longer possess the required UV-protective features of the parental molecules, which is prominently evident in several classes of filters: cinnamates; salicylates; camphor derivatives; and dibenzoylmethane derivatives [10, 16-18].

The photo-isomerization of UV filters is both a fast and reversible process, when in aqueous solution, giving origin to a mixture of *E* and *Z* isomers in equilibrium. In the environment, UV filters will always be found in either of these two isomeric forms, given the existence of carbon-carbon exocyclic double bonds in their structure.

However, commercial formulations of these compounds contain solely the *E* form of the compounds, despite their immediate photo-isomerization into the *Z* form upon exposure to UV radiation [19].

Another notable disadvantage of these reactions is, as approached earlier, apart from the loss of UV-protective capabilities, the production of potentially more troublesome by-products. For instance, the isomeric forms of UV filters might be chiral and therefore enantiomers, with similar physical-chemical properties, but the compounds will display very distinct environmental fate, behaviour and ecotoxicological profile. Contrary to the biological processes of degradation, that might be stereo-selective or enantio-selective [20], these abiotic processes are apparently not enantio-selective [19]. In light of this, the stereo-isomer composition of UV filters in natural waters seems indeed paramount in order to understand the compounds' fate and behaviour in the environment, but seldom has the theme been the subject of serious and focused investigation [3].

Díaz-Cruz [3] reviews the only existing study focused specifically on the subject: Buser [21] studied the chirality of MBC, showing that the stereo-isomer composition of the filter depended in fact on biological degradation occurring in waste water treatment plants, other water bodies like rivers or lakes, as well as plant or animal life.

There are numerous other studies on the photo-degradation or photo-stability of UV filters in general. One of the most important and popular UV filters, EMC, has been the subject of several interesting studies [10, 22-25]. In a study already mentioned, Sakkas [15] investigated the photochemical behaviour of the filter EDP in different water samples (sea water; swimming pool water; and distilled water) and under natural or artificial solar radiation. Results demonstrate that the filter degrades photo-chemically, originating several by-products; influence of dissolved organic matter (DOM) was also evaluated, showing that its presence decreases the photo-degradation reaction rates, since it competes with the filters for the incident photons; several by-products were also successfully identified. Huong [24] studied the photo-isomerization of EMC under artificial solar radiation and in several different solvents. Results showed significant loss in UV-absorbance capacity after irradiation, occurrence of chemical environmentally-dependent photo-isomerization $E \rightarrow Z$ as well as irreversible degradation of the filter structure; *Z* isomer displays considerable lower UV-absorbance capacity; and photo-degradation by-products were also detected and successfully identified. Pattanaargson [22] also approached the photo-

isomerization of EMC in different solvents and under natural solar radiation. The relevant results were as follows: photo-isomerization $E \rightarrow Z$ resulted in significant loss of UV-absorbance capacity; E - Z equilibrium in solution does occur but it depends on solvent polarity. Pattanaargson and Limphong [23] approached the photo-stability of EMC on a chromatographic basis, in order to determine the obtained photo-degradation by-products. The authors have successfully determined one photo-degradation by-product, identified as the Z form of the filter, referring that after one day of irradiation, approximately half of the amount of the original E form of the filter had been transformed into the by-product. No irreversible compound structure degradation of the filter was detected. Maier [26] carried out a spectroscopically-focused study on the spectral alterations undergone by a set of sunscreen products upon exposure to artificial solar radiation, and its reflection on the UV-absorbance capacity. Results have shown the following: loss in UVB-absorbance capacity never exceeded 5% and considering all the irradiation times; UVA-absorbance capacity loss was generally much more significant and frequent; all products displayed increased spectroscopic photo-instability at increasing wavelengths. Gaspar and Maia Campos [27] evaluated the *in vitro* photo-stability of different combinations of UV filters in sunscreen products, under artificial solar radiation. Results have demonstrated that the interaction between filters within a formulation influences their photo-stability; and formulations containing the filter OCR increased their UVA-absorbance capacity. Huong [28] carried out another study similar to the one mentioned before for EMC [24], but focused now on BDM [28]. As far as the results are concerned, BDM demonstrated photo-instability in non-polar solvents, with significant alterations in its absorption spectra; these alterations, despite significant, were found to be reversible after protection and storage of the irradiated solutions in the dark, but also found to be inhibited depending on the solvent conditions; the general behaviour of BDM was considered analogous to that of EMC, and it displayed quite significant and irreversible degradation of the filter structure in aqueous solution; several photo-degradation by-products were detected and successfully identified; photo-degradation in general was found to be significantly influenced and dependent on the medium and experimental conditions.

Many other similar studies exist, like Mturi's and Martincigh's [29] that dealt with the photo-stability of BDM in different solvents; or Hojerová's study [30], that ascertained the protective efficiency of several sunscreen products containing different UV filter formulations, and concluded that the sunscreen products' UV-protective

efficiency is quite distinct from one another, even between commercial products with the same labelled sun protection factor (SPF); Rodil [25] also evaluated the photo-stability of several UV filters, as well as the eco-toxicological profile of their photo-degradation by-products in aquatic microorganisms; Perugini [31] carried out a very interesting study on the effect of nanoparticle encapsulation of the filter EMC on its photo-stability; and Scalia [32] evaluated the effect of the natural antioxidant quercetin, on the photo-stability of a combination of two of the most popular UV filters used worldwide, EMC and BDM.

Degradation Induced by Disinfecting Agents

The water disinfection process has the fundamental purpose of destroying aquatic microbiological organisms, which represent the ultimate contagion source of disease. This is contrary to the concept of sterilization, which involves complete destruction of every microorganism, something that may not always be achievable or even necessary or beneficial [33].

For more than a century, chlorine has been the most popular disinfecting agent used worldwide, successfully controlling and even eliminating water-borne infectious diseases altogether [8]. Despite this, there are several other types of less popular disinfecting agents, like ozone or even UV radiation, both used in high-scale swimming-pool water disinfection, but also bromide-based water disinfecting agents, used in lower-scale swimming pools [33]. Although the removal of relevant pathogens and microorganisms is rather effective, the removal of DOM is not. Removal of organic pollutants is rather complex and varies significantly [3].

The disinfection process transforms the organic compounds in the water, giving origin to the so-called disinfection by-products (DBPs), or in this particular case, chlorinated DBPs. Over the years, chlorinated DBPs have been directly associated with several and serious potential toxicological effects, which forced authorities to consider the problem of production of these compounds in the context of drinking water disinfection process [3, 8]. Reports indicate that exposure to chlorinated DBPs might be directly associated with the occurrence of several cancers in human vital organs, so Gopal [33] approached predictive models for production and kinetics of DBP formation, their health effects, removal techniques, and guideline implementation.

Given the use of other aforementioned water disinfecting agents, apart from the vastly more popular chlorine, based on either bromide or iodine, important focus is now

being given to brominated and iodinated DBPs. These compounds are reported to be significantly toxic, not only in the range of carcinogenicity, but also genotoxicity and cytotoxicity. Such compounds include iodo-acids (IAs), like iodo-acetic acid, bromonitromethanes (BNMs), iodinated-trihalomethanes (THMs), 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (best known by its historical name, Mutagen X or MX), halogenated-aldehydes, halogenated-amides, bromate, and many others [34, 35]. Also, a particular reason for concern is yet another by-product of chlorine disinfection, the production of chloramines, which are originated from the reaction of chlorine with ammonia. Research has indicated that chloramines potentiate the IAs and iodinated-THMs production and accumulation within the water compartments [8].

Researchers have previously approached the general pathways of introduction and movement of synthetic organic pollutants, into and throughout the environment, with emphasis on the aquatic compartments [6, 8]. Generally speaking, the mode of introduction of these compounds into the environmental compartments will very much depend on their pattern of use or application [8]. When in the environment, however, these are transformed, chemically, photo-chemically or biologically. Usually, these processes will lead to the compounds' structure breakdown and subsequent elimination, but at the same time degradation by-products may also be produced, often more persistent and toxic than the original structures [6, 8]. Figure 2 represents the pathways involved in the generic fate and behaviour of UV filters and corresponding by-products in the environment [6].

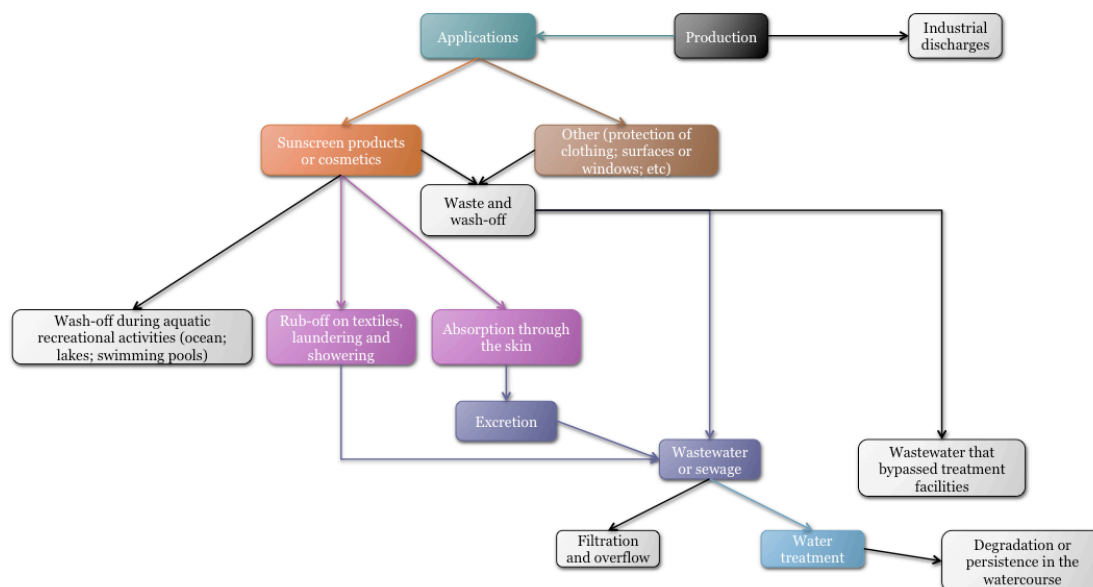


Figure 2. Relevant pathways of introduction of UV filters and their degradation by-products, into the environment (inspired by and adapted from [6]; boxes filled in light grey correspond to potential processes of release into the environment).

An evaluation on the occurrence of DBPs was carried out in Turkish superficial waters with low levels of dissolved organic carbon [36]. Results have shown that, given the susceptibility of the DBP precursors to associate with soils and sediments, events like ground- level run-off or leaching from soils as well as re-suspension into the watercourse from sediments, increased their levels in the superficial aquatic compartments. Upon treatment with chlorine disinfecting agents, it led to the production and increase of DBPs in drinking water.

There isn't any data available on the determination of UV filters DBPs in water treatment facilities [3]. Nonetheless, given the combined action of solar radiation and the presence of disinfecting species in solution, UV filters will be readily halogenated, giving origin to halogenated species of the parental compounds as well as many other DBPs as a result of the degradation of the original structure. Up to this moment, there are only four studies focused on the UV filter degradation reactions in aqueous solution on the presence of chlorine disinfecting agents [1, 37-39], along with a comprehensive review on both the UV filter photo-degradation by-products in aqueous solution, and the UV filters' DBP formation studies until 2012 [40].

Regarding the study of these compounds, very little is still known about the degradation reactions induced by disinfecting agents, and what is indeed known is focused solely on a very limited number of the most popular UV filters [1, 37-39]. More focus should be given towards the disinfection process and its implications on

the degradation of UV filters.

Eco-Toxicological Effects of UV Filters and Their Degradation by-products

The physical-chemical properties of UV filters, presented in Table 1, such as water solubility, vapour pressure and polarity, are crucial in order to determine their behaviour in the environment. As already mentioned, data points to their substantial tendency to concentration and accumulation within the environment and food chain, which, associated with their also reported significant potential for eco-toxicity, is quite problematic [3, 6, 8, 15, 41, 42].

Díaz-Cruz and Barceló [43] have reviewed some of the most important existing eco-toxicological studies, performed both *in vitro* and *in vivo*. What follows, is a brief summary on these and some additional relevant studies and subsequent results and conclusions.

***In vitro* studies**

Several UV filters have been reported to display estrogenic activity *in vitro* [7, 44-48]. The *in vitro* models applied or used on these and other studies, often revolve around the highly efficient, sensitive, fast and inexpensive recombinant yeast assay [44, 47, 48], MCF-7 breast cancer cells [45, 48], the human embryonic kidney 293 reporter gene assay (HEK293) [7], the human endometrial Ishikawa cell line [46], or rat and human primary hepatocytes [46].

Regarding the UV filters commonly approached in *in vitro* studies, benzophenones seem to be quite popular in that regard, with Schultz [44] investigating their estrogenic activity in specific, although the compounds have been addressed in many other studies, in particular BZ3 [7, 45, 47].

MBC has also been the subject of some particular studies, regarding its interaction towards estrogenic receptors [46, 48], amongst other more generic *in vitro* eco-toxicological studies [7, 45, 47]. Amidst the most popular UV filters, one must also underline the UV filters EMC, BDM and EDP, also investigated as to their estrogenic activity [7, 45, 47].

As for the relevant eco-toxicological conclusions arisen from these studies, all have emphasized the issues subsequent to this context of investigation. These problems include noncommittal results or the clear inability of the general *in vitro* models to account for the toxico-kinetics and toxico-dynamics of complex whole organisms,

which highlights their prominent limitations as to relevant predictive value for a mammalian *in vivo* context. Overall, nearly all filters have demonstrated dose-dependent estrogenic activity, with benzophenones being amongst the most active, as was the case of BZ3 [7, 45], with EMC [7] and BDM [45] being the least active and inactive, respectively, in the same type of studies. The estrogenic activity seems to be directly linked to the presence of benzene rings substituted with polar functional groups, particularly in *para*- position, as well as with the presence of symmetry in the molecule [44]. MBC, one of the filters studied in specific, has demonstrated either estrogenic activity analogous to that of other known weak estrogens [46] or ambiguous results occurring at extremely high levels [48].

Possible effects on thyroid hormonal regulation have also been mentioned [43, 48], but the data on the subject are rather scarce [49]. This study used a new human recombinant thyroid peroxidase stably transfected into a human follicular thyroid carcinoma cell line (FTC-238), in order to assess the possible effects of the filter BZ2.

Very significant disturbance of thyroid hormone homeostasis by inhibition of thyroid peroxidase was reported, making BZ2 the most potent thyroid peroxidase inhibitor found to date [49]. A similar study using human FTC-133 thyroid carcinoma cells [50] showed the opposite results for EMC and MBC.

Evaluation of mere estrogenic activity of UV filters has always been the main focus of hormonal activity studies of these compounds, but multiple combined hormonal effects (estrogenic, anti-estrogenic, androgenic and anti-androgenic effects) have seldom been investigated. Several UV filters have been recently shown to display multiple endocrine-disrupting behaviour like MBC, which displayed estrogenic and anti-estrogenic activities, or BZ3 and HS, which demonstrated estrogenic, anti-estrogenic and anti-androgenic activities [7, 51-53].

In another study [54], out of 19 UV filters and two benzophenone metabolites, all displayed some kind of hormonal effect, merely two (P25 and PAB) did not demonstrate multiple hormonal effects, while the vast majority demonstrated multiple effects. However, as Díaz-Cruz and Barceló argue [43], these effects might be subjective, since individual activities are directly and significantly dependent on the type of tests conducted.

Regarding the *in vitro* studies on UV filters' transformation or degradation by-

products, seldom has it been the subject of any studies. The exceptions are a study by Butt and Christensen [12], which dealt with the toxicity of photo-degradation by-products of EMC and BDM in a mouse lymphoma cell line (L5178Y-R), and a more recent study from Nakajima [38], which focused on the mutagenic activity of EMC and EDP using a mutagenic assay on a *Salmonella typhimurium* strain (TA100).

Butt and Christensen [12] demonstrated that exposure to irradiated solutions of UV filter resulted in increased cell mortality, independently of the irradiation time. Regarding Nakajima's study [38], both EMC and EDP were not mutagenic in the referred assay, with the opposite being exhibited after chlorination. EMC's mutagenic by-products of chlorination however, proved to be unstable after 6 hours of completion of the chlorination reactions, since the mutagenicity of the solutions decreased subsequently.

In vivo studies

Díaz-Cruz and Barceló [43] mention that benzophenones are the essential focus of study, regarding *in vivo* investigations, and these are mostly centered on evaluation of hormonal effects, effects on reproduction or fertility [54-64].

The usual *in vivo* models of investigation include mostly fish, particularly juvenile or mature fathead minnows (*Pimephales promelas*) [54-58], Japanese rice fish (*Oryzias latipes*) [58, 62] or rainbow trout (*Oncorhynchus mykiss*) [58], with some existing studies also in ovariectomized rats [59, 60, 61], tadpoles [57, 63] or even fetal rats [64]. Benzophenones are indeed a special focus of these studies, particularly BZ2 [56, 59, 60] and BZ3 [58, 60], given the fact that the molecular structure of this class of compounds is quite similar to other known estrogenic chemicals. But camphor derivatives have also been approached often, with particular emphasis on MBC [57, 62-64] and 3BC [55, 57, 63, 64]. There are two existing studies on the extremely popular EMC [61, 62].

Regarding the conclusions of these studies, all have emphasized concerning considerations, and in general, dose-dependent: significant bioaccumulation factors [55, 56], which has also been approached in previous chapters (see Table 1 and corresponding references [3, 6]); quite significant decrease in fecundity or complete cessation of reproductive ability [55-58]; demasculinization of secondary sexual characteristics [55, 57]; significant induction of Vitellogenin [54, 55, 57, 58]; prominent effects in the masculine and feminine gonad histology [55, 56, 57]; and development

of both oocytes and spermatocytes, as well as egg production, inhibited [55, 56, 57, 58].

On the other hand, MBC and 3BC displayed no accountable effects on tadpole's hormonal and thyroidal systems during metamorphosis [57], which was described as a critical stage quite susceptible to endocrine disruptions. The filter Ethyl-*p*-aminobenzoate (yet another PAB derivative) also did not exhibit negative effects on fathead minnows' weight and length development, and mortality was not verified either, upon exposure to the compound [54].

As a counterweight to all the concerning findings, some authors argue that many of the toxicological effects are found and reported at extremely high levels, sometimes as high as 75 fold the levels previously reported for wastewater effluents [58]. At environmental levels, however, MBC and 3BC were both studied as to their effects on the hormonal, thyroidal and sexual systems of tadpoles, during metamorphosis, and after 35 days of exposure, no relevant negative effects were found [63]. Contrary to this fact, studies have also reported that significant toxicological effects may indeed be found at low levels, as was the case with 3BC, which induced prominent histological and reproductive effects in fish and at low concentrations [57].

FUTURE PERSPECTIVES

In light of what was approached in this review, it is important to emphasize that very little is still known about the aqueous degradation reactions of UV filters induced by disinfecting agents. Very few filters have been studied in this context (essentially EMC, BDM and EDP), and considering the findings of these studies, it is imperative to focus even more on this context and extend the investigation towards other filters amongst the most popular. Special attention should also be given to the determination of their DBP's, which is a field of special concern given their potential hazardous toxicological effects.

Considering the eco-toxicological reviews, it seems clear that additional studies are also required, given the ambiguous results obtained so far. Both the *in vitro* and *in vivo* contexts of toxicological investigation are of paramount importance, and should therefore be used in succession in order to achieve a reliable assessment of the eco-toxicity of UV filters and by-products. However, there are still many issues to consider and solve, particularly regarding the *in vitro* models: the typical models used

carry significant unclarity regarding procedures and protocols; many display limited predictive value towards the *in vivo* results; and none reflect or replicate the metabolic processes of complex whole organisms, which is clear from the usually conflicting *in vitro* and *in vivo* results.

Naturally, the *in vitro* models lack the capacity to account for the toxico-kinetics and dynamics of complete organisms, which represents a comprehensive disadvantage. This fact stresses the need to carry out studies *in vivo*, following the investigations made *in vitro*, for these often generate inconclusive results and that do not necessarily reflect what may indeed occur in whole organisms.

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1.1.2. Pharmaceuticals

Contrary to personal-care products, which are meant to simply improve the quality of life, pharmaceutical compounds are more invaluable, as they represent a vital tool for diagnosis and treatment of human and animal diseases or afflictions. However, ordinary pharmaceuticals are now recognized as a significant source of nefarious toxicological implications to the natural, aquatic compartments and all the life forms contained within them [1, 2, 3].

Perception is now clear that pharmaceuticals are completely spread throughout the different environmental compartments, and since they are of vital importance, it means they are continuously prescribed to both humans and animals and subsequently released into the soils and superficial, aquatic courses [1, 4]. These aspects constitute reason for serious concern due to the significant potential of these compounds for environmental persistence, bioaccumulation and disturbing eco-toxicological profiles [1].

The pathways of inclusion of pharmaceuticals into the environment, are very similar to those presented for UV filters in the previous sub-section, but these display some important distinctions [5]. Figure 1 presents these paths in overall. Hundreds of pharmaceutical ingredients and related degradation compounds or metabolites have

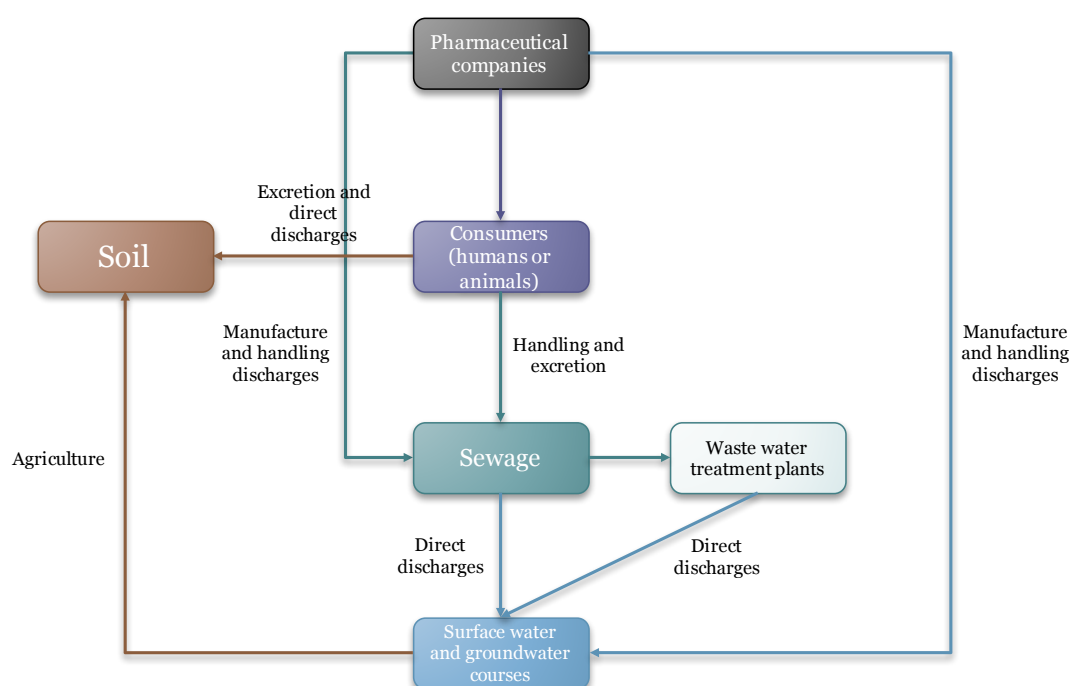


Figure 1. Pathways of inclusion of pharmaceuticals into the environment. Adapted from and inspired by [5].

been detected worldwide, consequence of the complexity and globalization of these paths of inclusion, namely in surface waters and discharge effluents but also in the soil, groundwater courses and drinking waters [1, 6]. Figure 1 may render a simplified outline of the processes that lead to the entrance of these pollutants into the environment, but those are slightly more intricate. The pharmaceutical companies, whilst producing the commercial products, handle hundreds of active ingredients, which, in turn, may be included into the discharge/sewage system [5-7]. Consumers, both human (private citizens or hospital institutions) and animal (farm animals in agriculture or domestic animals through veterinary services), will then ingest the products, yielding their natural transformation in the organism. This represents a form of inclusion of degradation by-products into the environment, which, as previously approached, are compounds that may display more concerning eco-toxicological features than the parental structures. Additionally, animal pharmaceuticals must also be highlighted for two specific reasons: first, pharmaceuticals applied in agriculture will be released into the soil, where they will therefore accumulate and may even be incorporated into the life cycles of plants [5, 8]; second, aquaculture must also be mentioned, for any pharmaceutical applied in this context will result in direct inclusion into the surface water courses. The municipal water treatment infrastructure and its disinfecting procedures are the obvious process for elimination and removal of pharmaceuticals. However, first, they also yield the formation of DBPs (addressed in the previous sub-section), which are also troublesome compounds on their own; and second, these methods have proven inefficient in completely eliminating the pollutants from the wastewater. In fact, this procedure has shown to merely remove about 20-80% of pharmaceuticals. In other words, significant amounts of pollutants will always remain in the water course, being subsequently released into other compartments, such as rivers, lakes, and even infiltrating into groundwater reservoirs [5]. Improving the efficiency of this elimination process, may be achieved with the incorporation of additional techniques and treatment, such as membrane filtration, activated charcoal and ozonation [1, 9, 10].

Table 1. Physical-chemical properties of some of the most popular pharmaceuticals. Adapted from [11].

Pharmaceutical	Action	pK_a	$\log K_{ow}$	$\log K_{oc}$	$\log K_d$
Ibuprofen	Anti-inflammatory	4.5-5.2	4	1.82-3.12	1.00-1.78
Carbamazepine	Anti-epileptic	13.9	2.45	2.26-3.48	1.31-1.83
17 β -estradiol	Estrogen	10.4	3.9-4.0	3.13-3.69	2.30-2.83
17 α -ethinylestradiol	Estrogen	10.4-10.7	4.2	2.0-4.16	2.08-2.85
Estrone	Estrogen	10.4	4.1	3.00-4.18	2.18-2.77
Sulfamethoxazole	Antibiotic	5.6-5.7	0.89	2.06-3.47	0.77-1.79
Naproxen	Anti-inflammatory	4.2	3.2	2.00-3.00	1.03-1.71
Diclofenac	Anti-inflammatory	4.15	4.5	2.20-3.42	1.26-2.18

When in the environmental, aquatic compartments, pharmaceuticals typically experience natural processes of removal, elimination or mitigation from the surface waters, as mentioned in section 1.1. The efficiency of these mechanisms, although, is directly correlated with the compounds' physical-chemical properties [such as the dissociation constants (pK_a), the octanol-water partition coefficients ($\log K_{ow}$), the organic carbon sorption coefficients ($\log K_{oc}$), the solid-water distribution coefficients ($\log K_d$), polarity and solubility] [11-13]. Table 1 presents the physical-chemical properties of some of the most common pharmaceuticals detected in surface waters [11]. There are cases of extremely popular pharmaceuticals like ibuprofen or acetaminophen, with higher octanol-water partition coefficients, and therefore, easily removed by sorption, reaching elimination rates higher than 90% [11, 14]. On the other hand, amide-based pharmaceuticals like carbamazepine, are known to display very low sorption rates below 30%. In cases of compounds such as the latter, physical-chemical properties render the pollutants extremely persistent in the surface waters, leading to possible infiltration and contamination of groundwater courses [11].

Biodegradation of pharmaceuticals is also a process to underline, even if it may be hindered by specific environmental circumstances, such as the prevalence of anaerobic conditions [11, 15, 16]. Surface waters' sediments are known to play a significant role in the removal of pharmaceuticals from the water phase, as was reported for the case of the compound oxytetracycline, found to have been transported into the sediment in more than 10 times the amounts detected in the water phase [11, 17]. Many pharmaceuticals may even be resistant to biodegradation, as it was shown for a series of pharmaceuticals, including ibuprofen, carbamazepine and acetaminophen, in river waters [14]. In other cases, such as caffeine, biodegradation is considered much more favourable and efficient, during waste water treatment [18].

The third process of elimination of pollutants in surface waters, is photo-degradation, that is, the transformation or degradation of compounds induced by

exposure to solar radiation (or more specifically, the UV portion of the radiation). Reports have shown that many pharmaceuticals, acetaminophen, ibuprofen and carbamazepine included, display very low rates of photo-degradation, which, in turn, may be increased with the formation of certain by-products that are distinctively more degradable [11, 14]. In the same trend, artificial UV radiation has also been shown to have very poor results in efficiently eliminating most of the known pharmaceuticals from drinking waters, with removal rates being often below 30% [19]. These combined studies seem to indicate that photo-degradation has very low significance to, or impact in the removal of pharmaceuticals in surface waters. In essence, with many compounds being resistant to the natural processes of elimination, and the latter being significantly inefficient in the quantitative removal of most compounds, it is safe to conclude that surface waters do have significant amounts of these pollutants and that these might pose a considerable threat to human, animal and the environment's health.

As for the risks and eco-toxicological effects that pharmaceuticals may have, the compounds are designated as biologically-active substances that affect the control mechanisms of living organisms, namely by regulating their metabolism or even by inducing hormonal changes or affecting hormonal balance, displaying maximum activity at low doses. These purposes are listed or directed specifically towards the

Diclofenac (anti-inflammatory)	- Caused the collapse of a population of wildlife vultures (<i>Gyps bengalensis</i>), due to renal failure [20]; - Induced strong afflictions of the kidney, liver and gills of laboratory subjects of rainbow trout (<i>Oncorhynchus mykiss</i>) [2];
17 α -ethinylestradiol (synthetic estrogen)	Led to the feminization of male subjects of fathead minnows (<i>Pimephales promelas</i>) in a lake, therefore causing the collapse of its population [21];
Sulfonamide (antibiotic)	Affected maize (<i>Zea mays</i>) and willow (<i>Salix fragilis</i>) plants in a greenhouse, leading to problems of root growth and high death rate of maize subjects [22];
Fluoxetine (anti-depressant)	Delayed the larvae development of laboratory subjects of leopard frogs (<i>Rana pipiens</i>) [23];
Ivermectin (veterinary parasiticide)	Affected laboratory and field populations of dung flies and beetles, leading to mortality of eggs and larvae [24];
Oxazepam (anxiolytic)	Altered the behaviour and the feeding rate of laboratory subjects of European perch (<i>Perca fluviatilis</i>) [25];
Eurofloxacin and ciprofloxacin (antibiotics)	Inhibited the growth of laboratory subjects of cyanobacterium (<i>Anabaena flosaquae</i>) and duckweed (<i>Lemna minor</i>) [26];

Figure 2. Eco-toxicological implications verified in the laboratory and in the natural environment, due to direct exposure to some of the most popular pharmaceuticals. Adapted from and inspired by [5].

target subjects, that is, humans or animals. However, being released into and accumulating in the superficial, aquatic compartments, these pharmaceuticals may induce nefarious changes or effects in aquatic organisms that lie outside of the scope of their intended action [5]. Figure 2 presents some of the most prominent reports on the establishment of a direct link between exposure to pharmaceuticals and unintended, negative effects on populations of non-target animals [2, 5, 21-26].

In brief summary, regarding the most significant eco-toxicological effects of exposure to pharmaceuticals discriminated on Figure 2:

- Diclofenac, an anti-inflammatory commonly prescribed for treatment of arthritis and pain in humans, was used in Pakistan to treat symptoms of fever in cows; when vultures started feeding on the carcasses of subjects from the treated group of cows, it caused severe kidney failure on the vulture population, ultimately leading it to near-extinction [20];
- A study conducted in the field, namely at the Experimental Lakes Area (ELA) in Ontario, Canada, concluded that chronic exposure of a fathead minnow fish population to low concentrations of 17 β -ethinylestradiol, the synthetic estrogen used in birth-control pills, caused an overwhelming rate of feminization of male subjects in the population, leading to the near extinction of the species from the lake [21];
- Exposure of European perch fish to dilute concentrations of oxazepam, a popular benzodiazepine anxiolytic compound, caused significant alteration of the subjects' behaviours, including increased activity, decreased social ability and increased feeding rate; these observations led the authors to safely conclude that exposure of fish populations to anxiolytic drugs may significantly alter their social and feeding habits as well as even promote nefarious evolutionary implications [25];

Other negative aspects of the eco-toxicological risks of exposure to pharmaceuticals, must be addressed. First, we have the potential synergistic effects of mixtures in the superficial water courses. Compounds that may be present at levels below the predicted no-effect concentrations (PNEC), may display highlighted toxicity enhanced by the presence of and subsequent interaction with additional pharmaceuticals in solution. The case of the interaction between non-related compounds carbamezapine, a common anti-epileptic, and clorofibric acid, a lipid lowering agent, proves such toxicity enhancement [27]. The interaction of both,

displayed significantly increased toxicity towards *Daphnia magna*, compared to the individual exposure to the compounds, under the same concentrations [27]. Additionally, another growing concern of pharmaceutical exposure, regards the potential for the creation of even more antibiotic-resistant strains, in natural colonies of microorganisms. These super-bacteria, incredibly resistant to many of the current strongest antibiotics, represent a significant threat to the efficient treatment of several infectious diseases induced by these organisms [28]. Reports have confirmed the connection between exposure to popular antibiotics found in superficial water courses, including ciprofloxacin, tetracycline, ampicillin, trimethoprim and erythromycin, and the increase of the antibiotic resistance of naturally-occurring bacteria [29]. Even trace levels of antibiotics have been found to yield such effects on bacteria [30].

As previously mentioned, pharmaceuticals display a considerable proclivity towards persistence in the aquatic, superficial compartments, and it is known that in the case of diclofenac, the popular anti-inflammatory, levels have been detected significantly above the predicted no-effect concentrations (PNEC) in several countries around the world [5]. This is indeed concerning, since several of the effects portrayed or reported in this section have been displayed at trace levels, which raises the question of how significant or damaging will the effects be, at concentrations well above such threshold. This fact, together with the perception that compounds may present increased levels of toxicity due to interactions with other pharmaceuticals, should promote further research into the fate and behaviour of such compounds in the aquatic environments, as well as their eco-toxicological risks to non-target organisms and the ecosystems in general.

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Chapter 2 - Degradation studies of selected UV filters in aqueous solution

2.1. The UV filter 3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate (IMC)

This study was entirely carried out and fulfilled by the author, Albano Joel Moreira Santos, with supervision, guidance and revision being provided by Professor Dr. Joaquim Carlos Gomes Esteves da Silva.

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Fate and behaviour of the UV filter 3-methylbutyl-(2*E*)-3-(4-methoxyphenyl)-acrylate (IMC) in aqueous solution

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Abstract

The disinfection-induced and photo-induced degradation of the UV filter 3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate (IMC) is investigated. High performance liquid chromatography was used to follow the degradation reactions, determine kinetic features and also the influence of external variables on degradation [pH; temperature; chlorine concentration; dissolved organic matter (DOM) concentration]. Liquid chromatography preceded by solid-phase extraction, was used for the determination of by-products. IMC was found to rapidly and substantially degrade in aqueous solution, when in exposure to active chlorine, displaying a kinetic rate constant of $0.015 \pm 0.002 \text{ s}^{-1}$ and a half-life period of 46.2 ± 6.2 seconds. Contact with aqueous active chlorine proved to yield merely the dichlorinated form of IMC, as the prominent disinfection by-product. The assessment of influence of external variables on degradation, was insured by a Box-Behnken experimental design formulation approach, with factorial analysis. In this regard, data shows that temperature plays a decisive factor in the reaction rate, without DOM in solution, leading to decreasing degradation rates as the temperature increases. With DOM in solution, pH and DOM proved to be the most influential variables on IMC degradation. Regarding the photo-degradation study, results have shown that IMC remained quite stable in aqueous solution when exposed to artificial solar radiation, with a rate constant of $0.00073 \pm 0.00002 \text{ min}^{-1}$ and a half-life period of 938.5 ± 31.1 minutes. This work addresses yet another filter that has never been studied and hopes to provide clarification on its environmental fate and behaviour when in the aqueous environmental compartments.

Keywords

IMC; 3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate; isoamil *p*-methoxycinnamate; isopentyl 4-methoxycinnamate; active chlorine; disinfection by-products; DBPs.

1. Introduction

Emerging pollutants (EPs), a class in which ultraviolet (UV) filters are included, are a concerning group of compounds, since, by its own definition, little is still known about their fate and behaviour in the aquatic environmental compartments, and so they are not bound by existing water-quality legislation [1]. Structurally speaking, these compounds display the typical characteristics of priority organic pollutants (POPs), with the addition of a significant proclivity towards persistence and accumulation within the environment and food chain [2-5].

In extension to their found environmental prevalence and bioaccumulation, there are also rising concerns regarding their toxicological and reactive profiles in aqueous solution [1, 3, 6]. The parental compounds are linked to several and potential ecotoxicological implications [4], being also acknowledged to degrade when in contact with active chlorine [7], yielding chlorinated by-products [better known as disinfection by-products (DBPs)] with potential toxicological effects of their own [1].

UV filters' environmental levels are generally established as residual, often considered too low to reflect any serious eco-toxicological problems. But considering the fact that their paths of inclusion into the aquatic compartments are extremely complex [3], it is very likely that they might in fact accumulate to the point of actual effect concentrations, as well as possibly augmenting the toxicity of other compounds also found in solution [4]. As Díaz-Cruz and Barceló [4] have concluded, several UV filters have been shown to particularly display hormonal or anti-hormonal activity, with some even showing multiple-hormonal activity, and half-maximum effect concentrations have been also concluded to be similar to those of other familiar hormonally-active pollutants. Additionally, relevant to the issues just outlined, are the concerns regarding the questionable efficiency of wastewater treatment plants (WWTPs) in quantitatively removing the more polar UV filters from the inflowing water courses [8, 9], which may mean that we can be exposed to higher amounts of these compounds through our drinking water.

The degradation of UV filters has seldom been approached, and very few studies have thoroughly and comprehensively delved into the two existing degradation sources, disinfection-induced and photo-induced. Negreira and collaborators [9, 10] only focused on disinfection-induced degradation, providing kinetic features and DBP determination on compounds such as 2-thylhexyl-4-(dimethylamino)-benzoate

(EHPABA), benzophenone-3 (BP-3), and benzophenone-4 (BP-4). Nakajima and co-authors [11] have also only approached the disinfection-induced degradation, evaluating the stability of 2-ethylhexyl-4-methoxycinnamate (EMC) and EHPABA as a function of pH and chlorine levels, but also providing an outline of their mutagenic potential as well as their DBPs. Sakkas and collaborators [12] have been the only researchers to address both degradation contexts, presenting their reaction kinetic features and attempting to identify potential by-products. Finally, Duirk and co-workers [13] have also only investigated the disinfection-induced context, focusing essentially on the kinetic features of the degradation of BP-3 and benzophenone-8 (BP8), whereas Grbović [14] only presented a mass spectrometry identification of 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate's (DHHB) DBPs, without focusing on the reaction itself.

As far as the experimental approach is concerned, complex environmental impact assessment is predicated on the existence of solid and dependable analytical methods, able to determine compounds at levels as close as possible to ng/L [12]. Up to 2006, there were around 90 publications dealing with UV filter determination, and mostly, in complex cosmetic product formulations [2]. Chisvert and Salvador [2] also highlighted a very significant problem with UV filter-related research: the need for expansion of the number of filters studied or approached, since most seem to deal with the same handful of compounds, whilst there are more than 40 filters currently being used in, and regulated by the EU, USA and Japan. The analytical techniques often implemented on UV filter research are fundamentally chromatographic, given the fact that the vast majority of filters has organic nature and are usually present in complex matrices, requiring therefore a separation procedure. More importantly, within the scope of chromatographic techniques, liquid chromatography (LC) is by far the most employed, since UV filters are not volatile compounds [2].

The purpose of this work is to address some of the most prominent problems in this field, which is the limited framework of UV filter study: in number, with the few existing works only focusing on a handful of compounds, when there are several dozens of filters of which nothing is effectively known concerning their fate and behaviour in aqueous solution; and also in content, since practically every work available only approaches the disinfection-induced degradation without providing a comprehensive outline of the complex and potential influence of external variables (like pH, temperature, and chlorine or dissolved organic matter levels), while also disregarding the photo-degradation significance. In this work, the reactivity of a new filter, 3-

methylbutyl-(2*E*)-3-(4-methoxyphenyl)-acrylate (IMC), is thoroughly addressed, in aqueous solution and in both the degradation scenarios, chlorine-induced and photo-induced. More specifically, the aim was to provide the most expansive and elaborate reactivity assessment, by using high performance liquid chromatography coupled with ultraviolet diode-array detection (HPLC-UV-DAD) to accurately follow the degradation reactions, through direct sample injection at different reaction times. Additionally, the influence of pH, active chlorine concentration, water temperature and dissolved organic matter (DOM) concentration, was evaluated experimentally, in a complex design formulation, allowing a meticulous insight on the evolution of the degradation rates as a function of the studied variables. Photo-degradation was ensured by artificial irradiation with a solar radiation simulator with air mass filters and by using a liquid light guide. Subsequently, all transformation by-products were studied using a highly sensible liquid chromatography coupled to ultraviolet diode-array detection and mass spectrometry (LC-DAD-MS) system, with a quadrupole ion trap for more accurate and precise identification of compounds. To increase reliability on the detection and identification of compounds, solid-phase extraction (SPE) was always carried before analysis, in order to foment the increase in chromatographic peak intensities.

2. Materials and methods

2.1. Reagents

The UV filter IMC (CAS registry number 71617-10-2 and molecular formula $C_{15}H_{20}O_3$) was purchased as an analytical grade standard from Sigma-Aldrich, Merck, Darmstadt, Germany. A reagent grade sodium hypochlorite (NaOCl) solution, with 3.5% active chlorine labelled content, was purchased from VWR Chemicals, Radnor, PA, USA. From this, a stock solution of 432 mg/L in active chlorine content, was prepared and permanently stored at 4°C and removed only upon use. Active chlorine content was evaluated by means of the standard iodometric titration method. The compound L-Ascorbic acid at 99% was purchased from Merck, Darsmtadt, Germany. Deionised water (conductivity < 0.1 μ S/cm) was the only water source in every experiment. The pH was always regulated and adjusted with the addition of ordinary standard solutions of either hydrochloric acid (HCl) or sodium hydroxide (NaOH). Pre-concentration of the aqueous reaction mixtures, necessary for the analysis and identification of by-products, was carried out by solid-phase extraction (SPE) using LiChrolut EN 200 mg,

6 mL standard cartridges from Merck, Darmstadt, Germany. All organic solvents used for pre-concentration or chromatographic purposes, namely methanol, acetonitrile or dichloromethane, were of liquid chromatographic grade and purchased from Merck, Darmstadt, Germany. Dissolved organic matter (DOM) was obtained from pinewood soil [15-17].

2.2. Chromatographic conditions

2.2.1. HPLC-UV-DAD

High performance liquid chromatography coupled with ultraviolet diode array detection (HPLC-UV-DAD) was used to follow the IMC degradation reactions along with time. This system was composed of an isocratic pump (Hewlett–Packard 1100 Series, Böblingen, Germany), a manual sample injection valve with a 20 μ L loop (Rheodyne 7725i, Rohnert Park, USA), a silica-based C18 reverse phase column [Dionex Acclaim™ 120 C18, particle size 3 μ m, pore diameter 175 Å (4.6 $\times$ 150 mm), Thermo Fisher Scientific, Sunnyvale, CA, USA] and a photo-diode array detector (UV 6000LP with a 50 mm LighPipe flow cell, Thermo Fisher Scientific, Sunnyvale, CA, USA). The mobile phase used to investigate the degradation reactions, was a mixture of liquid chromatography-grade acetonitrile and deionised water (80:20, v:v), at a constant 0.60 mL/min flow rate. Before being used, mobile phases were always filtered by pressure through a borosilicate glass funnel (pore size 4-8 microns). UV absorbances were monitored and recorded at a total scan mode (200-600 nm), and the entire system was controlled with the Xcalibur 1.4 SR software.

2.2.2. LC-DAD-MS

Liquid chromatography coupled with diode-array detection and mass spectrometry (LC-DAD-MS), was used to determine the potential by-products of all the degradation reactions. The system was composed of a HPLC pump (Finnigan Surveyor LC Pump Plus), an auto-sampler (Finnigan Surveyor Auto-sampler Plus) and a photo-diode array detector equipped with a LightPipe flow cell (Finnigan Surveyor PDA Plus Detector) (all instrumentation was purchased from Thermo Electron Corporation, Waltham, USA), coupled with the same reverse phase silica-based column [Dionex Acclaim™ 120 C18, particle size 3 μ m, pore diameter 175 Å (4.6 $\times$ 150 mm), Thermo Fisher Scientific, Sunnyvale, CA, USA]. The mass spectrometer was a Finnigan LCQ Deca XP Max (Thermo Electron Corporation, Waltham, USA) coupled to the LC

system and equipped with an electrospray interface as ionization source and a quadrupole ion trap for MSⁿ experiments. The system was operated in positive ion mode between $m/z = 150$ and 1000 , with the following conditions: spray voltage, 5.0 kV; capillary voltage, 15 V; and capillary temperature, 300°C . The device was programmed to perform a full mass scan first, and then a fragmentation analysis (MS/MS) of the most intense ion. The mobile phase was the same, although the deionised water included 0.1% of formic acid (HCOOH), in order to promote the ionization of the compounds of interest. The flow rate was the same as in the previous subsection, and the mobile phase was also always filtered prior to use. The system was controlled by using the Xcalibur 1.4 SR software, as well.

2.2.3. Performance of the analytical method

The performance of the analytical method was evaluated according to the ICH standard guidelines for the validation of analytical procedures [18]. The calibration was accomplished through the analysis of nine solutions between the range 0.02250 mg/L (9.060×10^{-8} mol/L) and 25.00 mg/L (1.006×10^{-4} mol/L), with three trials carried out for each. Correlation coefficients (R^2) were all determined to be higher than 0.996 .

2.3. Disinfection-induced degradation

2.3.1. Determination of reaction kinetics

Experiments were designed to be as simple as possible, with deionised water as the reaction solvent, at neutral pH, room temperature, in the dark and with constant stirring, in order to simulate a swimming pool water environment as much as possible.

A stock solution of IMC was prepared, by dissolving 750 mg of compound in 100.00 mL of absolute ethanol (final concentration being 7500 mg/L or 0.03020 mol/L). For every subsequent experiment, a new standard IMC solution was always prepared from the dilution of 100.00 μL of stock into 10.00 mL of methanol (final concentration being 75.00 mg/L or 3.020×10^{-4} mol/L).

Reactions were carried out in 250 mL borosilicate glass beakers, containing 0.5002 mg/L (2.014×10^{-6} mol/L) of IMC and 5.002 mg/L (6.716×10^{-5} mol/L) of NaOCl in 100 mL of deionised water, ensuring that the variance in the levels of active chlorine along with time remained negligible. Prior to the addition of active chlorine, the solution containing solely IMC was always analyzed by HPLC-UV-DAD, in order to get a

chromatographic record of the initial amount of compound. After this step, NaOCl was added, pH verified, monitored and regulated if necessary ($\text{pH} = 6.79 \pm 0.03$), and the water temperature recorded and monitored ($T = 19.3 \pm 0.2 \text{ }^{\circ}\text{C}$). An aliquot was removed from the mixture every 10 seconds, its residual active chlorine was quenched with the addition of 10 μL of an approximately 65 g/L standard ascorbic acid (AA) solution, and then analyzed by HPLC-UV-DAD, in order to allow the determination of reaction kinetics.

2.3.2. Identification of DBPs

The experimental conditions were similar to those described in the previous subsection. The reaction was left undisturbed for 60 minutes, and after this period of time, the entire solution was pre-concentrated through SPE (by using 6 mL, 500 mg, hydrophilic-lipophilic balance cartridges from Waters Corporation, Milford, MA, USA). Before the procedure itself, the cartridges were always conditioned with 3 mL of methanol and 3 mL of deionised water. This process was enhanced with the aid of a peristaltic pump (Minipuls® 2 from Gilson, Inc., Middleton, WI, USA). The analytes of interest, adsorbed in the cartridge filter, were then extracted with 3 mL of dichloromethane, subsequently evaporated with a gentle stream of nitrogen gas. The final residue was diluted in 500 μL of methanol and analysed directly by LC-DAD-MS.

2.3.3. Influence of external variables on degradation

In order to ascertain the influence of significant, external variables to the superficial aquatic environments, on the degradation of IMC, an experimental design formulation was created, with a posterior statistical analysis of variance (ANOVA). This process enabled the determination and outline of tendencies within the results. Analysis was processed by the Unscrambler® v9.2 (CAMO Process AS, Oslo, Norway) software program.

The study was based on two Box-Behnken designs with response surface methodology, one with and another without the inclusion of dissolved organic matter (DOM) as a design variable. General characteristics of both designs were as follows:

- First Box-Behnken set of experiments:
 - Non-design variable: IMC degradation percentage;
 - Design variables: [NaOCl]; pH; and temperature;

- 12 experiments (with 2 trials) + 1 central experiment (with 3 trials) = 27 experiments;
- Second Box-Behnken set of experiments:
 - Non-design variable: IMC degradation percentage;
 - Design variables: [NaOCl]; pH; temperature; and [DOM];
 - 24 experiments (with 2 trials) + 1 central experiment (with 3 trials) = 51 experiments;

More detailed information on the specific experimental conditions of both sets, are included in section 2 of the supplementary material.

2.4. Photo-degradation in aqueous solution

This part of the work was performed by means of artificial solar irradiation, using a solar radiation simulator (Newport Oriel 96000 150 W) with Air Mass Filters 1.5 Global 81094, and a liquid light guide, the tip of which was positioned approximately 1 cm above the aqueous solution.

A new standard solution was always prepared for every experiment, by transferring 100.00 μL of the stock into 10.00 mL of methanol (final concentration 75.00 mg/L or 3.020×10^{-4} mol/L). Experiments took place in simple borosilicate glass containers, as outlined in previous sections, with 100 mL of deionised water spiked with IMC to reach the concentration of 0.5002 mg/L (2.014×10^{-6} mol/L). An aliquot of the solution was always analysed prior to the irradiation process, by HPLC-UV-DAD and also in the UV-Visible spectrophotometer, in order to obtain a record of the initial amounts of compound as well as its initial UV spectrum profile. The irradiation process lasted for a period of 6 hours, and an aliquot was always removed at each hour period and analysed directly in the spectrophotometer and in the HPLC-UV-DAD system.

3. Results and discussion

3.1. Disinfection-induced degradation

3.1.1. Determination of reaction kinetics

As mentioned in subsection 2.3.1, IMC degradation kinetics was determined in aqueous solution, with IMC at 0.5002 mg/L (2.014×10^{-6} mol/L) and NaOCl at 5.002

mg/L (6.716×10^{-5} mol/L), at neutral pH ($\text{pH} = 6.79 \pm 0.03$), room temperature ($T = 19.3 \pm 0.2^\circ\text{C}$), in the dark and with constant stirring.

As it has been underlined in other publications [7, 9-13], namely Deborde and Von Gunten's comprehensive review on the degradation of organic and inorganic compounds with active chlorine in aqueous solution [19], these compounds are known to react with active chlorine in a global second-order kinetic profile, that is, first-order according to each reagent (organic compound and total active chlorine in solution). However, in the specific case of IMC and this study, it was found that the compound's degradation profile fits more accurately and adequately a pseudo-second order kinetic profile. In other words, the reaction kinetics will have a global third order profile, second order in regards to IMC and first order to active chlorine in solution. Thus, the kinetic reaction law will be:

$$v = -(\Delta[\text{IMC}]/\Delta t) = k_{\text{app}}[\text{Cl}_2]_t[\text{IMC}]^2$$

Where $[\text{IMC}]$ is the concentration of the UV filter, k_{app} is the apparent global third-order rate constant and $[\text{Cl}_2]_t$ is the total concentration of active chlorine species in solution ($[\text{Cl}_2]_t = [\text{HOCl}] + [\text{ClO}^-]$). Upon the conditions just outlined at the beginning of this section as well as subsection 2.3.1, we are left with an experimental context where the variation in active chlorine along with time is essentially negligible and therefore assumed as constant [7]. Consequently, the rate of the degradation reaction will depend solely on the concentration of the UV filter and the corresponding differential equation will be:

$$v = -(\Delta[\text{IMC}]/\Delta t) = k_{\text{obs}}[\text{IMC}]^2$$

Where k_{obs} represents the observed pseudo second-order rate constant, which includes the apparent global third-order rate constant and the total active chlorine concentration ($k_{\text{obs}} = k_{\text{app}}[\text{Cl}_2]_t$). Subsequently, the differential rate equation can be integrated, in order to obtain the corresponding integrated rate equation with its dependency of time:

$$1/[\text{IMC}] = 1/[\text{IMC}]_0 + k_{\text{obs}}t$$

Figure 2 represents the linear plot of $1/[\text{IMC}]$ as a function of reaction time, fitting, as expected, a linear degradation trend line. The determined observed pseudo-second order degradation rate constant for IMC was $0.042 \pm 0.001 \text{ L mol}^{-1} \text{ s}^{-1}$, and the half-life was 23.8 ± 0.6 seconds. These experiments showed that IMC degrades quite

significantly but also overwhelmingly fast, reaching half of its initial levels in about 24 seconds. The kinetic window for this exercise was ultimately set to 90 seconds, but experiments lasted longer, in order to ascertain the fate of the compound beyond that point. After this stage, the degradation rate did reach a plateau, with the amount of compound not lowering any further than 20% of the initial level. For comparison purposes, Nakajima [11] studied EMC, a compound of the same class of IMC, and he also concluded that EMC degraded quite substantially and rapidly, with its levels apparently reaching 20% of the original amount in about 60 minutes. More recently, in a work from our research group [7], EMC was indeed found to degrade quite substantially, reaching a 97% degradation rate in about 6 hours. In the present work, IMC did reach a similar plateau of 80% degradation but under 2 minutes of reaction time. Nakajima [11] determined the rate constant for EMC degradation to be $0.0095 \pm 0.0007 \text{ min}^{-1}$, with its half-life period being 72.7 ± 5.5 minutes. Consequently, the data implies that, despite the structural similarities between IMC and EMC, the reactivity of both compounds in aqueous solution with exposure to active chlorine, is very much distinct, with IMC proving to be about 90 times more reactive than its familiar compound EMC. Structurally speaking, the only difference between both compounds, is the size of the side-chain in the *para* position to the methoxy functional group. The methoxy group is a known electron donor, while the alkyl chain has an electron-acceptor character. This fact contributes to the electronic stabilization of both molecules, although, since the electron-accepting group in IMC is indeed shorter, this means that the electronic delocalization might be less efficient than in EMC, making it a more reactive compound.

In conclusion, these differences in reactivity and behavior of two compounds of the same class, underline, yet again, the need to consider more UV filters, including members of classes with compounds already addressed, because it is not accurate to assume or extrapolate fate and behavior characteristics simply based on similar structures.

3.1.2. Identification of DBPs

The LC-DAD-MS chromatogram of the degradation reaction, including the mass spectra of the corresponding peaks, are presented in Figure 3. More data on the identified peaks, is provided in Table 2, with retention times, estimated molecular formulas and the most prominent molecular ions obtained. Figure 4, provides an analogy between the UV absorption spectra of IMC and its dichlorinated DBP. Figure

5 presents the overall reaction and the most likely sites on the molecule to suffer the electrophilic substitution. Figures 6 and 7 present the proposed paths of fragmentation and the molecular structures yielded in that process. Additional information on the identification of the compounds, is present in section 1 of the supplementary material, including all the mass spectra of the compounds and their first and second fragmentations, in Figures S1 to S6.

Analysis by LC-DAD-MS identified merely one DBP of IMC, namely the dichlorinated form of the parent compound, at retention time 8.17 minutes of the chromatogram in Figure 3, as well as the expected and natural confirmation that IMC was represented by the peak at retention time 23.21 minutes. According to the comprehensive review work of Deborde and Von Gunten [22], on the chlorination of organic and inorganic compounds, active chlorine will react with an aromatic compound essentially by electrophilic substitution on the benzenic ring, and in *ortho* or *para* positions to a functional group, due to its activating role towards those specific sites. In the molecular structure of IMC (see Figure 1), the *para* position is occupied with a long side chain, leading to strong steric repulsions preventing the substitution in the *meta* position, so the electrophilic substitution will occur essentially at both the *ortho* positions regarding the methoxy group, as is presented in Figure 5. The reaction rate being quite significant, as shown in subsection 3.2.1, in parallel with the fact that only the dichlorinated form of IMC was detected and identified by LC-DAD-MS, demonstrates that the electrophilic substitution of chlorine is indeed rapid and sequential (the rate of substitution may even be highlighted by the electron-donor abilities of the methoxy group). Its also important to underline that no traceable amounts of monochlorinated compound were even detected, in a context of excess active chlorine. As for the MS results (see Table 2), the molecular ions detected for both peaks in the chromatogram of Figure 3, in positive ion mode ($m/z = 249$ for $[\text{IMC-H}]^+$, and $m/z = 318$ for $[\text{Cl}_2\text{-IMC-H}]^+$), were consistent with the molecular masses of both compounds (248.3175 g/mol for IMC, and 317.2070 g/mol for $\text{Cl}_2\text{-IMC}$). As for the fragment ions obtained (the most predominant are presented in Figures 6 and 7), there is no frame of reference with which to establish an analogy, but it is possible to align some of the most prominent in this case with the ones obtained by this research group for the compound EMC, namely the ions $m/z = 169$ and 213.

3.1.3. Influence of external variables on degradation

Natural and artificial, superficial, aquatic compartments, such as swimming pools or even lakes, are characterized by several important external variables such as water temperature, chlorine or DOM levels in solution, as well as pH. This section is focused on the investigation of the extent of their influence on the degradation rates of IMC, evaluated by means of a Box-Behnken, multi-factorial analysis with response surface methodology.

This study is divided in two models, one with and another without DOM in solution, in order to measure the behaviour of the system in the presence and absence of this variable. The details on the analysis of variance (ANOVA) on the two models, are presented in section 2 of the supplementary material, including the validation of the statistical model and the ANOVA tables provided.

The quadratic response surface equation obtained for the first model is the following:

$$\hat{Y} = 59.070 + 0.316A - 0.473B - 1.155C + 3.673AB - 0.445AC + 1.592BC + 1.232AA - 4.567BB + 0.304CC$$

With the variable A being NaOCl concentration, B representing pH, C is Temperature, and AB, AC, BC, AA, BB and CC are the corresponding quadratic interactions between variables. According to the results obtained in the first model, the most influential variables are the linear term water temperature (β -coefficient = -1.155 ± 0.299) and, to a larger extent, the quadratic term or interaction $[\text{NaOCl}] \times \text{pH}$ (β -coefficient = 3.673 ± 1.303). In subsection 3.2.1, it had already been defined that IMC displays a substantial reactivity towards active chlorine in solution, and at neutral pH, so this portion of the work was also important to realize which variable could impair or enhance the already high degradation rates of this compound. According to the relevant response surface obtained for this model, and presented in [Figure 8](#), higher degradation percentages are clearly obtained within acidic to neutral pH values and across the entire interval of chlorine concentration studied, with maximum values occurring either at lower chlorine concentrations and lower pH values, or higher chlorine concentrations and higher pH values. Hypochlorous acid (HOCl) is known to be the most prominent chlorine disinfection agent, predominating at acidic and neutral pH conditions. As for hypochloride anion (ClO^-), it is known as a much less efficient chlorine disinfection agent, and it prevails in alkaline conditions [19]. What [Figure 8](#) shows, as expected, is that the more efficient HOCl does not require high concentrations to effectively degrade the parental compound, although the degradation is always higher at acidic to neutral

conditions, regardless of the active chlorine concentration, due to the prevalence of this chlorine species in solution.

As for the second model, the obtained quadratic equation was:

$$\hat{Y} = 49.807 - 0.293A + 1.803B - 1.235C - 0.104D + 0.421AB - 1.270AC + 0.06720AD - 0.637BC - 0.193BD - 0.207CD + 0.491AA + 0.387BB + 0.874CC - 0.719DD$$

With A representing NaOCl concentration, variable B being DOM concentration, C is pH, D is Temperature, and AB, AC, AD, BC, BD, CD, AA, BB, CC and DD are the quadratic variable interactions. Results have shown that pH (β -coefficient = -1.235 ± 0.877), [DOM] (β -coefficient = 1.803 ± 0.358) and the quadratic interaction [NaOCl] x pH (β -coefficient = -1.270 ± 0.729), were the most prominent variables on IMC degradation. The most relevant response surface, detailing the degradation percentage of IMC as a function of the interactive variables NaOCl concentration and pH, is presented in [Figure 9](#). In the previous study, without DOM in solution, higher degradation of IMC was obtained at acidic-neutral pH values, across the different active chlorine concentrations, no doubt, due to the significance of the more efficient HOCl species on the degradation process. In this case, however, and with DOM in solution, the highest degradation percentages were found at higher active chlorine concentrations and at acidic conditions. This scenario was expected, although not exclusively around the highest active chlorine levels. There were also significant degradation percentages obtained at lower chlorine concentrations and alkaline conditions. These findings were not expected, since ClO^- is much less prominent in the degradation process than HOCl, and more so at lower active chlorine concentrations. The results may be a consequence of the action of DOM, found to be a significant independent variable on itself, which might have enhanced the susceptibility of IMC towards attack by the usually less efficient hypochloride anion. Regarding the independent significance of DOM on degradation, results show that higher degradation values were displayed at higher DOM levels in solution. DOM is known to play a very important role on the degradation of synthetic organic compounds [\[6\]](#), in the natural, aquatic, environmental compartments. This effect of DOM enhancing the degradation rates of IMC was not entirely unforeseen, but it was somewhat expected that the degradation rates in each experiment would slightly decrease due to the active competition for available chlorine. However, the opposite was somewhat found, and this effect might be explained in light of the overwhelming reactivity between IMC and available chlorine in solution. DOM's role in this study, it seems, was only

significant enough to influence IMCs reactivity towards the different active chlorine species, according to the solution pH.

3.2. Photo-induced degradation

3.2.1. Determination of reaction kinetics and photo-stability assessment

Photo-induced degradation was conducted using the experimental conditions outlined in subsection 2.4. By using 250 mL borosilicate glass vessels, containing 100 mL of deionised water spiked with IMC to reach the concentration 0.5002 mg/L (2.014×10^{-6} mol/L), with constant stirring and continuously exposed to artificial solar radiation, aliquots were removed from the reaction vessel after each hour of irradiation, and then directly analysed in the UV-Visible spectrophotometer and in the HPLC-UV-DAD system. Prior to irradiation, the reaction sample was always analysed both in the spectrophotometer and also in the HPLC-UV-DAD, in order for the initial IMC spectroscopic conditions and concentration to be recorded for subsequent analysis. Figure 10 presents the normalized exponential plot of IMC concentration along with time of exposure to artificial solar radiation, and Figure 11 represents the UV spectra of the reaction sample prior to, and after 6 hours of irradiation.

As is visible by the analysis of Figure 10, IMC photo-induced degradation follows a first-order kinetic plot line, yielding a kinetic rate constant of $0.00074 \pm 0.00002 \text{ min}^{-1}$ and a half-life period of 938.5 ± 25.4 minutes. Studies carried out by this research group on EMC, a familiar compound of IMC, showed that EMC degraded more rapidly than IMC through exposure to artificial solar radiation, generating a first-order kinetic profile with a rate constant of $0.0046 \pm 0.0004 \text{ min}^{-1}$ and a half-life period of 150.4 ± 12.0 minutes. These results demonstrate that IMC, the compound on which this work is focused, is considerably more photo-stable than its familiar UV filter, EMC. As was approached in subsection 3.1.1, less significant electronic delocalization would theoretically render IMC a more reactive compound, but that's not the case. On the other hand, EMC was also shown to significantly isomerize from its commercial form *E* to the photo-isomer *Z*, which was not detected in the case of IMC. The photo-isomerization of EMC could mean that the isomers could act as photo-sensitizers and enhance the global photo-degradation reaction, therefore yielding a more significant kinetic rate and a lower half-life period for that compound. As for the spectroscopic findings, results presented in Figure 11 demonstrate a significant loss of UV absorption

capacity throughout the total period of irradiation. IMC displays its peak of UV absorbance in the UVB range, specifically around 306-309 nm, and the maximum absorbance of the compound shows a total decrease of around 57% of its absorption capacity after the total period of irradiation. Results obtained by this research group for the familiar compound EMC, have showed similar findings, with EMC displaying again more photo-instability than IMC, with more than 90% loss in UV absorption ability, after 6 hours of irradiation.

3.2.2. Determination of photo-induced by-products

For this part of the work, reaction conditions were similar to those of the previous subsection. In this case, though, the reaction was left undisturbed for 5 to 6 hours and only then analysed by HPLC-UV-DAD, for preliminary assessment of potential by-products, subsequently pre-concentrated by SPE (in a similar way to the one described in subsection 3.2.2), and then studied by LC-DAD-MS, for identification of compounds. [Figure 12](#) presents a total ion current (TIC) chromatogram obtained by LC-DAD-MS, corresponding to an experiment carried out during 5 hours of artificial irradiation. In the chromatogram, one can perceive the IMC peaks, prior to the start of the irradiation process, and after 5 hours of irradiation (300 minutes). In the previous subsection, it was already concluded that IMC displays significant photo-stability, and these experiments confirmed that assessment, as expected. No additional peaks were, in fact, detected after the total irradiation time, including the known $E \rightarrow Z$ photo-isomerization reaction already reported to the familiar compound EMC [\[21-23\]](#). The important conclusion, is that IMC is indeed substantially photo-stable, through the course of the reaction time, to generate any traceable amounts of the Z photo-isomer. With a photo-degradation half-life period of 938.5 ± 25.4 minutes, one could possibly expect to obtain such results after that period of total irradiation. For technical and safety reasons, however, the artificial irradiation device could not remain functioning uninterrupted for such a long period of time, without complete and thorough supervision. Sequential irradiation was carried out for 6 hours per day, for 3 days, but results didn't change, since, as its also been reported [\[24\]](#), photo-isomerization of UV filters is often a reversible process. Thus, it is safe to conclude that IMC remains quite photo-stable in aqueous solution, when exposed to artificial solar radiation for long periods of time, without any relevant or measurable traces of photo-isomerization and effective photo-degradation.

4. Conclusions

In aqueous solution, and in a context of excess of active chlorine, IMC was found to be overwhelmingly reactive in a pseudo second-order kinetic profile, displaying a degradation rate constant of $0.042 \pm 0.001 \text{ L mol}^{-1} \text{ s}^{-1}$, and a half-life period of 23.8 ± 0.6 seconds. The electrophilic substitution of chlorine was therefore found to occur rapidly and sequentially, in the two *ortho* positions to the methoxy functional group in the benzenic ring, yielding only the dichlorinated form of the parental compound as a DBP. The most significant variable interactions on degradation of IMC, as expected, was found to be the active chlorine concentrations and pH. Without DOM in solution, higher degradation rates were found across all active chlorine concentrations evaluated, and only within the acidic-neutral pH range, as expected, highlighting the strong role of the more efficient HOCl on degradation. With DOM in solution, the highest degradation rate was found at the highest active chlorine concentrations and at lowest pH values, with significant degradation also at the lowest chlorine levels and at alkaline conditions. In this context, DOM apparently played a significant role in enhancing the reactivity of IMC towards the less efficient ClO^- species. In general, the overall comprehensive reactivity of IMC towards active chlorine proved to be significant enough not to be slowed by the competitive role of DOM towards chlorine in solution, and therefore, it seems, the variable ended up highlighting the degradation of the compound itself. As far as photo-induced degradation is concerned, IMC displayed very significant photo-stability but degraded slowly nonetheless, by means of a first-order kinetic model, with a degradation rate constant of $0.00074 \pm 0.00002 \text{ min}^{-1}$ and a half-life period of 938.5 ± 25.4 minutes. No photo-degradation by-products were detected, including the known $E \rightarrow Z$ photo-isomerization reaction already reported for the familiar compound EMC. This fact is explained either by the significant photo-stability of the parental compound itself, not generating any traceable amounts of by-products, or the possible instability of the latter.

To conclude, there are currently more than 40 UV filters regulated and applied worldwide, the crushing majority of which remain unapproached in any given meaningful study on the context of reactivity towards active chlorine and (natural or artificial) solar radiation. The focus of current environmental research in general, should try to converge more actively in assessing the fate and behaviour of even more compounds of this group, in the natural and anthropogenic aquatic environmental compartments. Results of the present work, have concluded that IMC is incredibly

reactive towards active chlorine in aqueous solution, yielding chlorinated by-products of the parental compound in the process. Other DBPs, like halogenated methanes or acetic acids, are also known to be originated in these types of reactions. Additionally, exposure to UV radiation also promotes the photolysis and photo-isomerization of UV filters, leading to the formation of free radicals and other reactive intermediates, as well as photo-isomers, which contrary to the original compounds, will display unwanted UV absorption and eco-toxicological profiles. Therefore, it becomes increasingly relevant for environment research to keep its focus on these compounds and their fate and behaviour, but also, parallel to the study of existing compounds, for the pharmaceutical research to try to create new and potentially more appealing UV filters. New UV filters should be focused on the strengthening of its structural features, shielding these compounds against reactivity towards active chlorine, subsequently reducing the potential for the formation of DBPs, which carry serious and damaging eco-toxicological profiles. Naturally, increasing the new compounds' resistance to photolysis and photoisomerization, should also be a priority. Photo-induced reactive particles, such as free radicals, are formed from UV filters included in sunscreen formulations, which are applied on the human skin. More importantly, these highly reactive particles are known to exhibit the potential to penetrate the skin and damage cells and tissues, which is a significant cause for concern.

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Figures

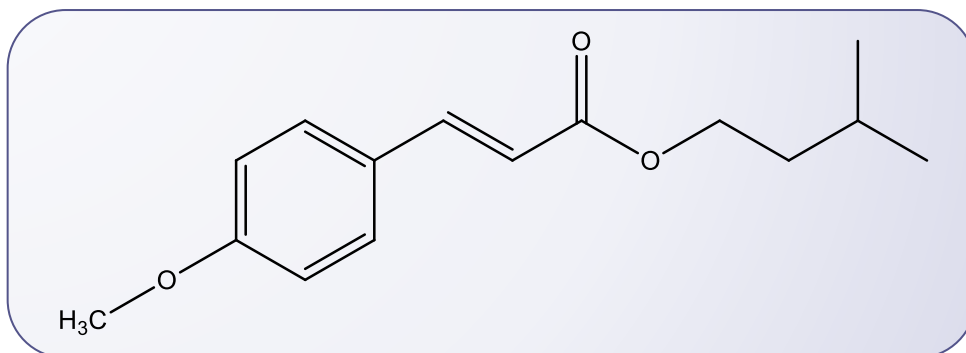


Figure 1. Structure of the UV filter isoamyl-*p*-methoxycinnamate (IMC).

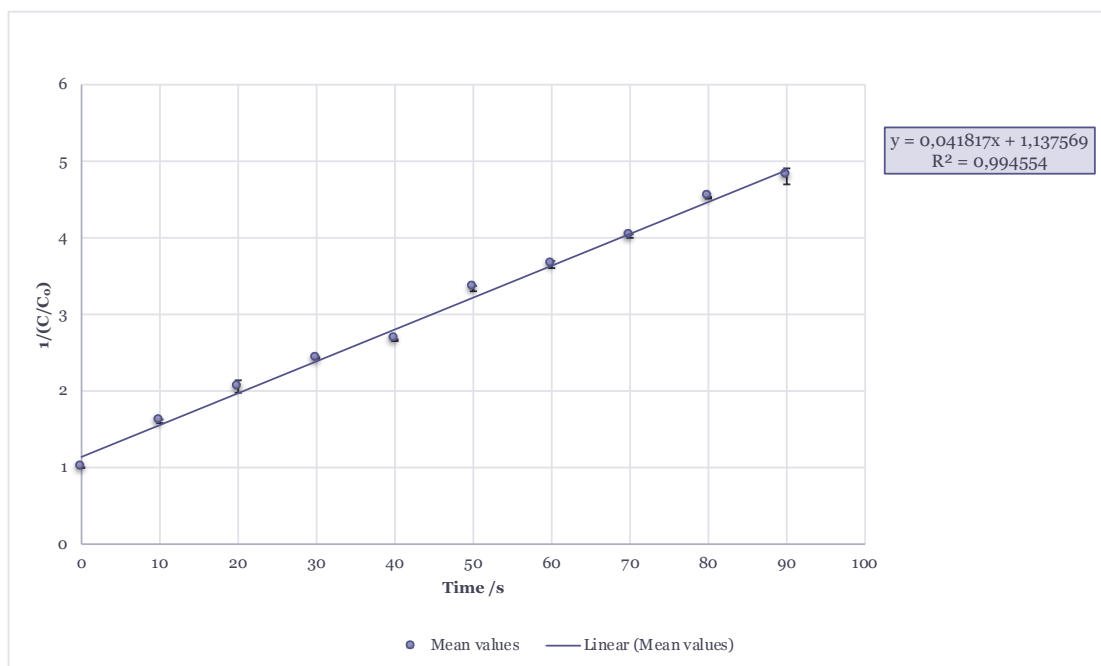


Figure 2. Pseudo-second order linear plot of the inverse of the normalized IMC concentration as a function of reaction time, in aqueous solution, at neutral pH and room temperature.

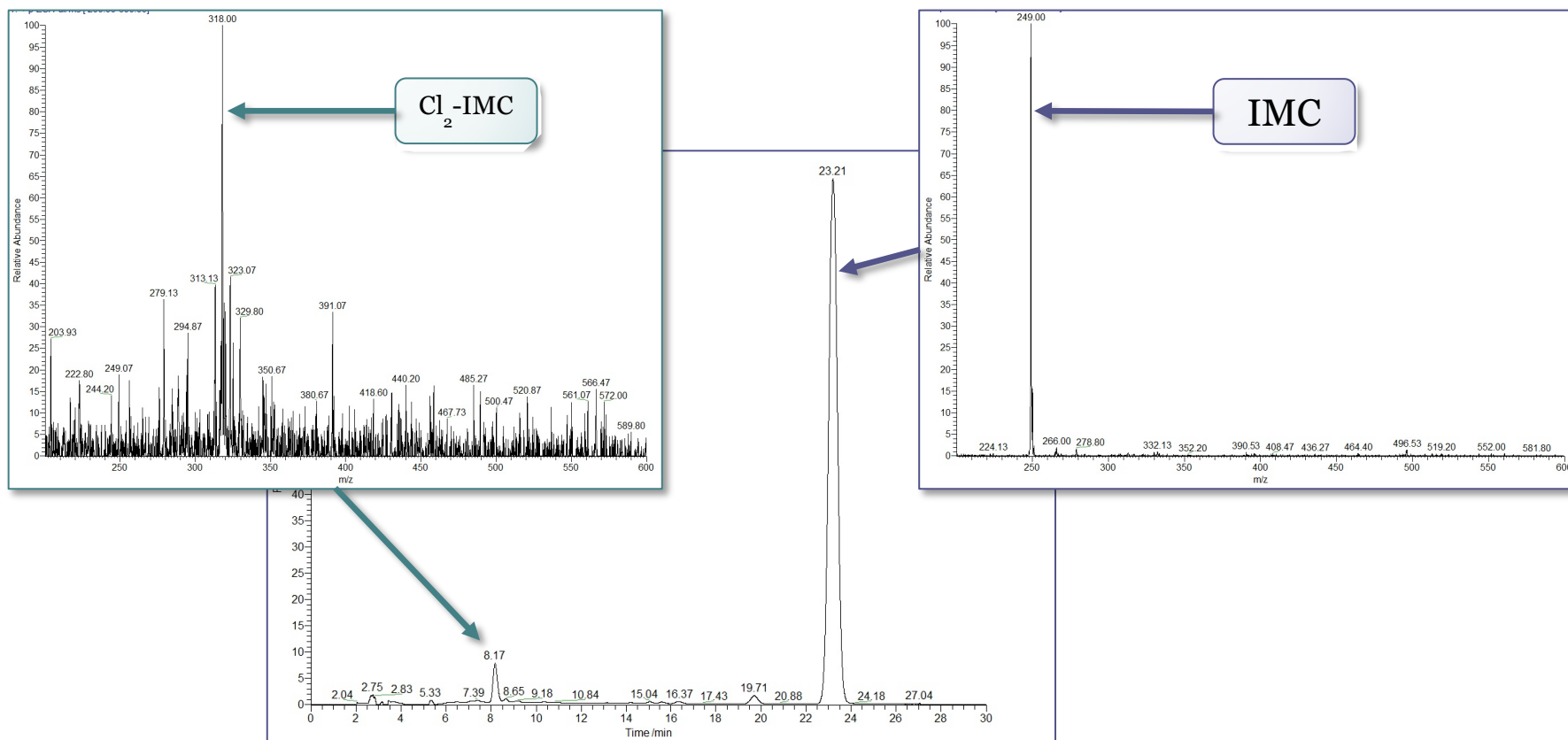


Figure 3. LC-MS-DAD total ion current (TIC) chromatogram of the degradation reaction with peaks successfully identified by MS, and corresponding mass spectra.

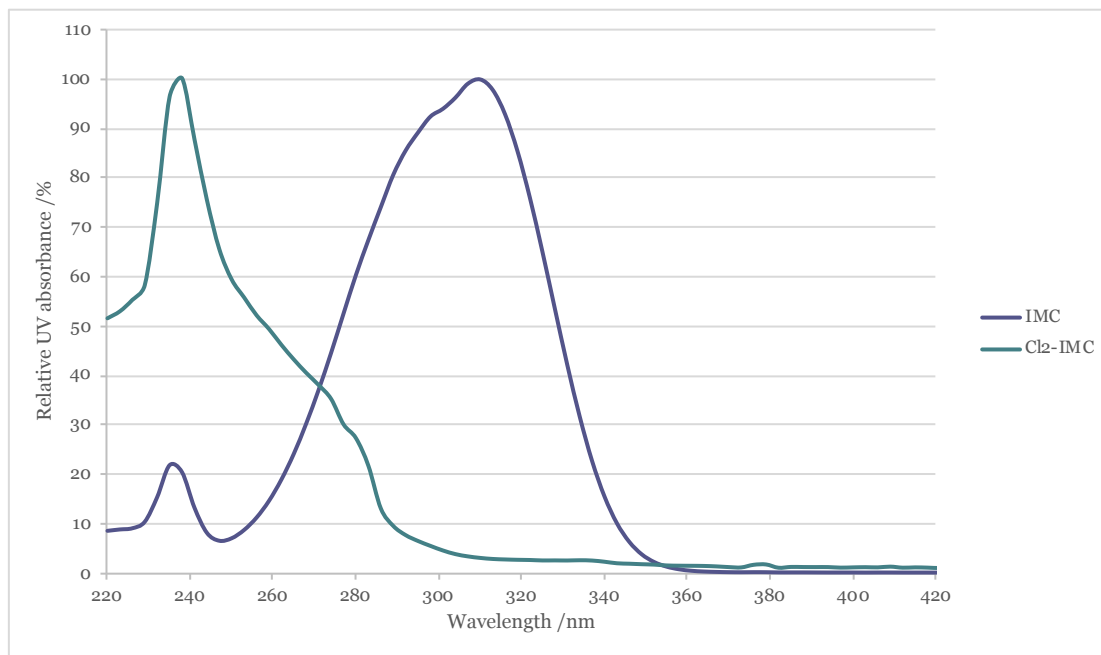


Figure 4. UV absorbance spectra of the parental compound IMC, and its dichlorinated DBP, Cl₂-IMC.

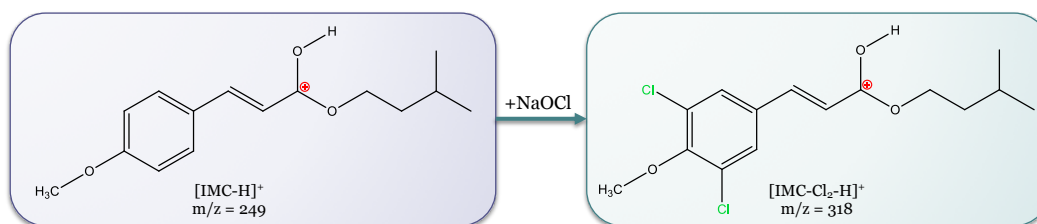


Figure 5. Electrophilic substitution of active chlorine into the *ortho* positions to the methoxy functional group.

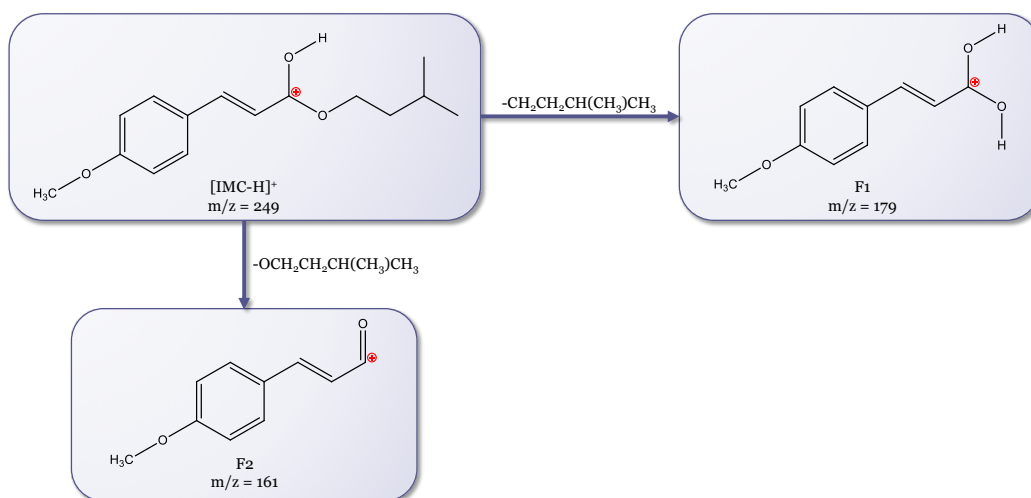


Figure 6. Estimated molecular structures for the most prominent fragment ions obtained for IMC.

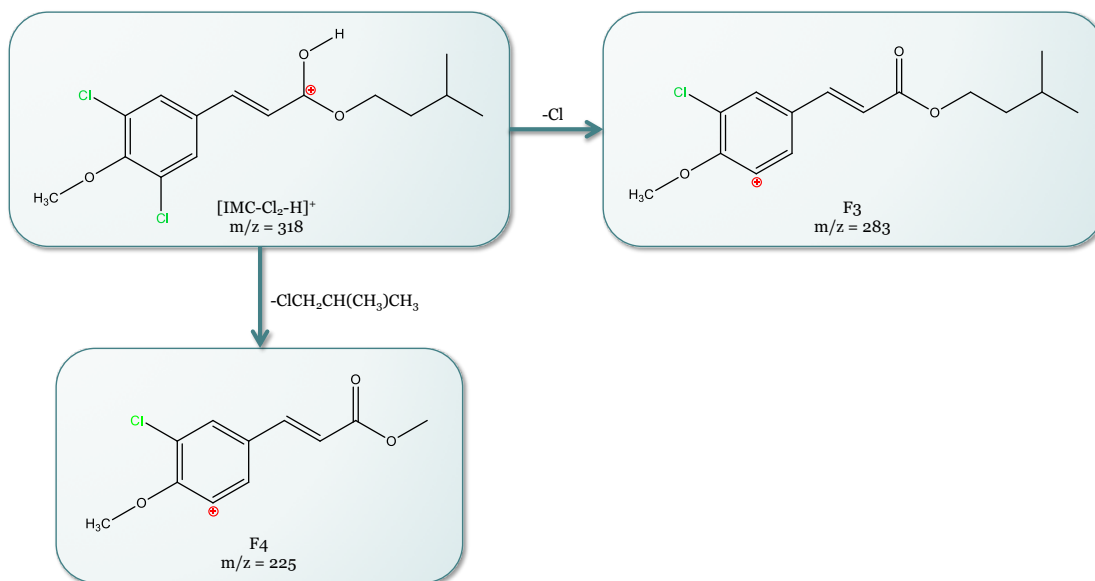


Figure 7. Estimated molecular structures for the most prominent fragment ions obtained for $\text{Cl}_2\text{-IMC}$.

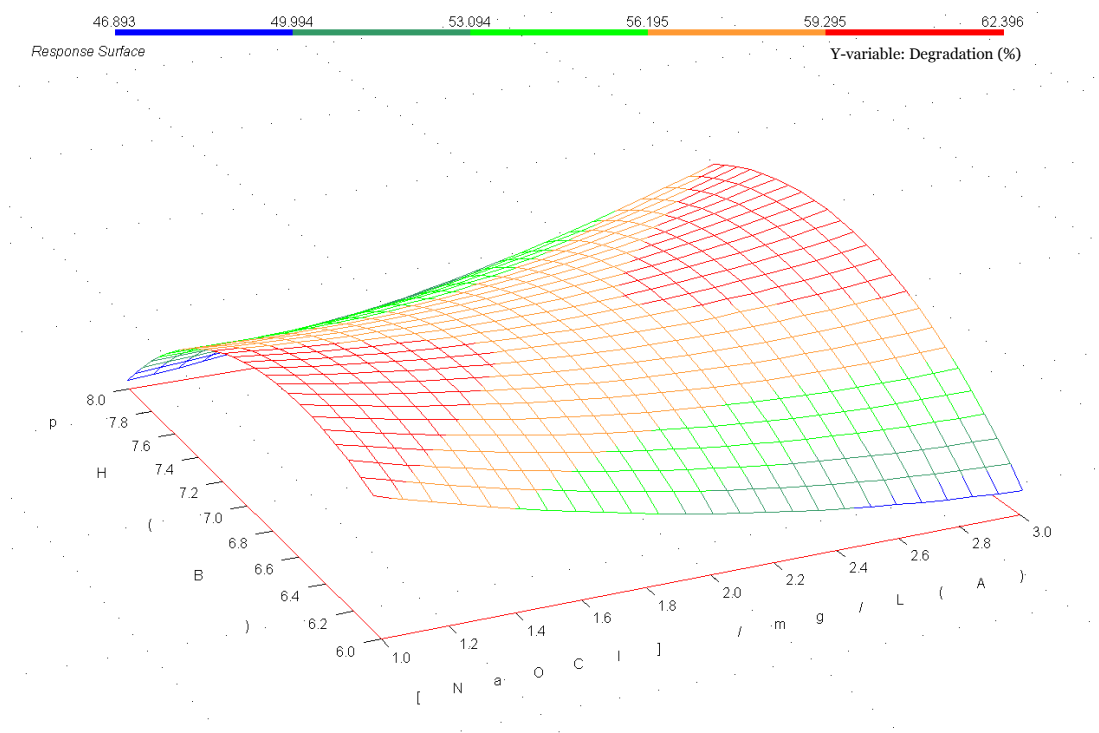


Figure 8. Response surface detailing the IMC degradation percentage obtained as a function of the interactive variables NaOCl concentration and pH, in the first model (without DOM in solution).

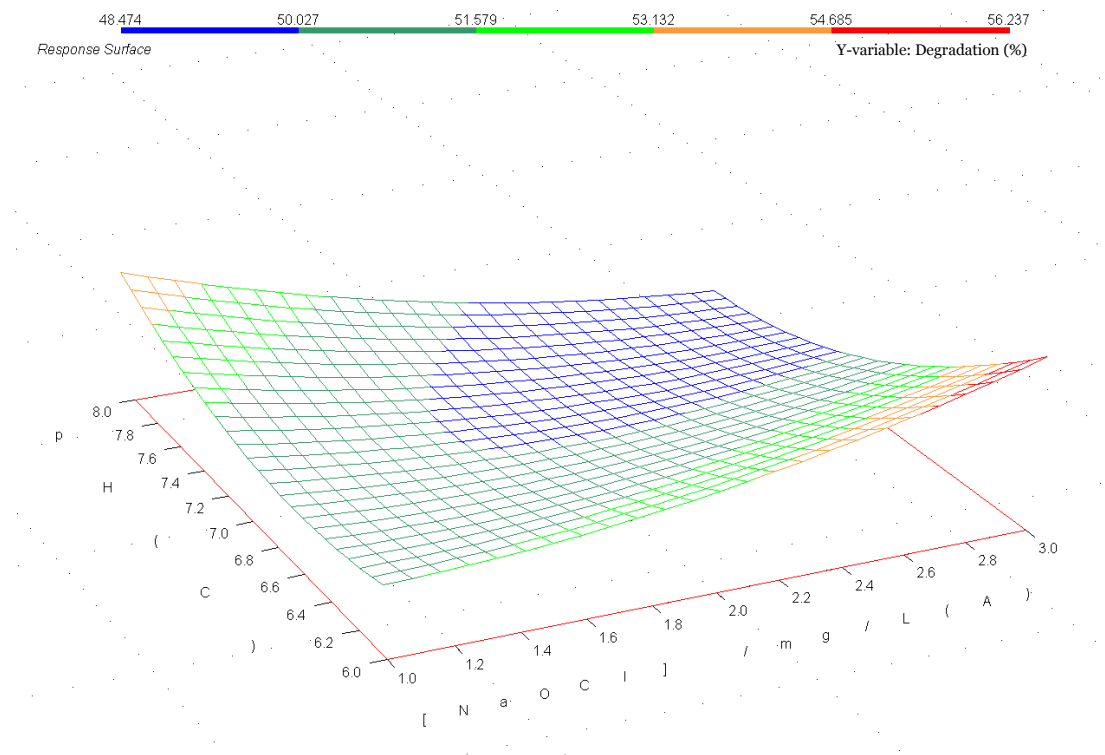


Figure 9. Response surface detailing the IMC degradation percentage obtained as a function of the interactive variables NaOCl concentration and pH, in the second model.

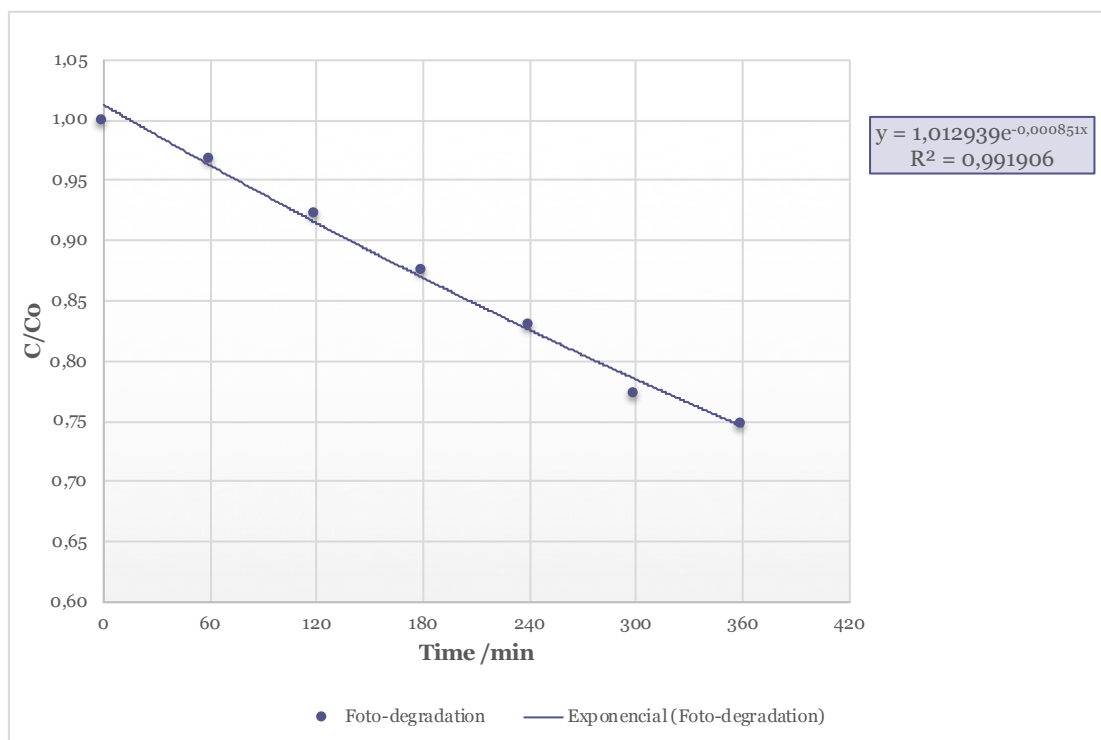


Figure 10. First-order exponential plot of the normalized IMC concentration as a function of reaction time, in aqueous solution and during exposure to artificial solar radiation.

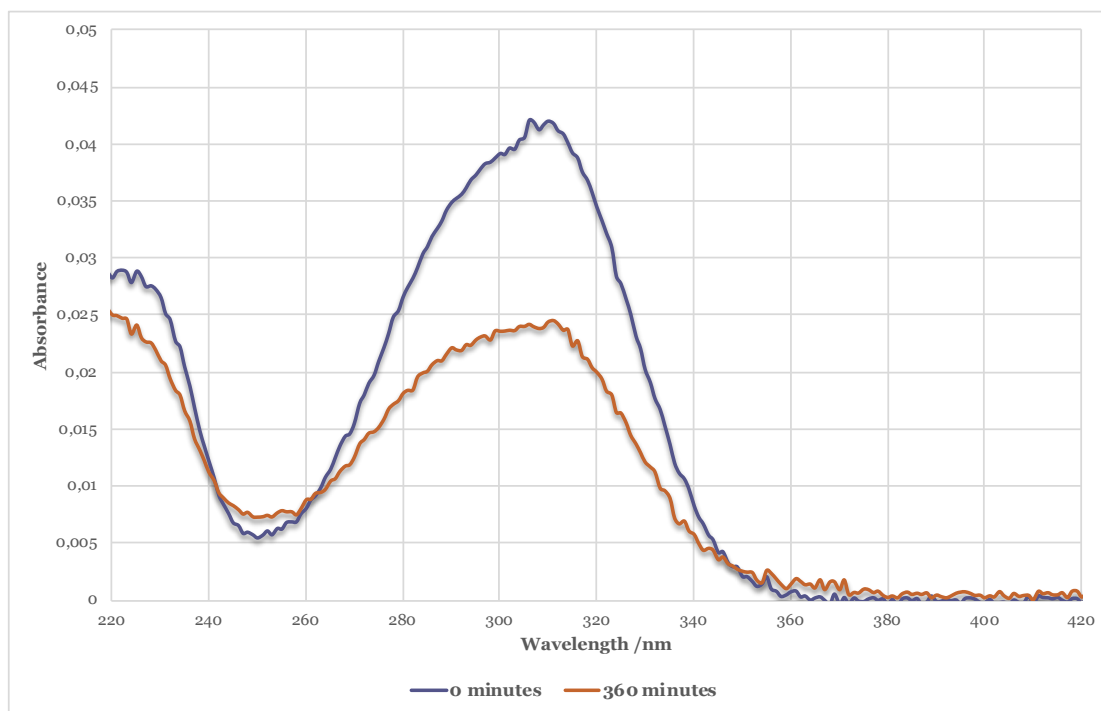


Figure 11. UV spectra of IMC aqueous reaction mixture prior to and after 6 hours of exposure to artificial solar radiation.

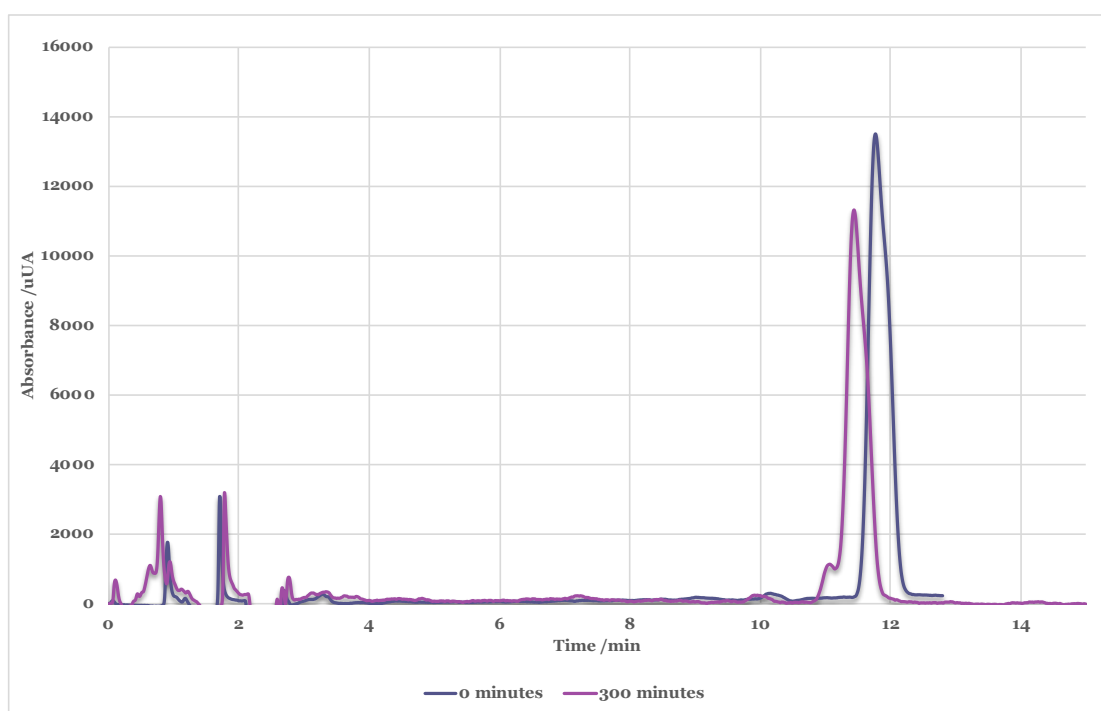


Figure 12. LC-MS-DAD total ion current (TIC) chromatogram of the photo-degradation reaction with peaks corresponding to the reaction mixture prior to artificial solar radiation exposure, and after 5 hours of irradiation.

Tables

Table 1. Physical-chemical properties of IMC.

IUPAC name	Acronym	Regulation	UV range	$\lambda_{\text{máx}}$ /nm	ϵ /L mol ⁻¹ cm ⁻¹	Molecular mass	Log k_{ow} †	Solubility* /g/l
3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate	IMC	The filter is currently regulated in the EU, Australia, New Zealand, South Africa and Japan	UVB	307-308	24.335	248.3175	4.06	0.06

* Determined in water and at 25 °C, according to [6].

† Octanol water partition coefficient (k_{ow}); represents the ratio of the concentration of a substance in octanol and the concentration of the same substance in equilibrium in water, at a certain temperature.

Table 2. LC-MS-DAD data for the compounds successfully identified in the study.

Retention time /min	Identification	Molecular ion /m/z	Fragmentation ions /m/z (relative intensity /%)	Proposed molecular formula	Abbreviation
8.17	[IMC-Cl ₂ -H] ⁺	318.07	282.53 (100); 224.47 (42); 102.13 (38); 299.73 (34) 201.20 (100); 169.13 (42); 213.07 (34);	C ₁₅ H ₁₉ O ₃ Cl ₂ ⁺	Cl ₂ -IMC
23.21	[IMC-H] ⁺	249.07	179.07 (100); 161.07 (20);	C ₁₅ H ₂₁ O ₃ ⁺	IMC

Supplementary material

Fate and behaviour of the UV filter 3-methylbutyl-(2*E*)-3-(4-methoxyphenyl)-acrylate (IMC) in aqueous solution

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1. Identification of DBPs

The molecular structures proposed for the most prevalent fragment ions detected in the case of IMC and its dechlorinated DBP, Cl₂-IMC, are presented in [Figures 6 and 7](#) of the main manuscript. The mass spectra for both compounds, as well as their first and second fragmentations, when applicable, are presented in this supplementary document, in [Figures S1 to S6](#). Identification details are as follows:

- Peak at 8.17 minutes (identified as the dichlorinated form of IMC, Cl₂-IMC): mass spectrum yielded the m/z value of 318.07 (100%), consistent with the molecular mass of the compound (317.2070 g/mol);
 - The first fragmentation of this peak originated the m/z values of 282.53 (100%), 224.47 (42%), 102.13 (38%) and 299.73 (34%);
 - The second fragmentation of the peak yielded the m/z values 201.20 (100%); 169.13 (42%); 213.07 (34%) and 154.87 (23%);
- Peak at 23.21 minutes (identified as the parental compound, IMC): the mass spectrum of this peak showed a m/z value of 249.07 (100%), consistent with the molecular mass of monochlorinated DHHB (248.3175 g/mol);
 - First fragmentation of this peak yielded the m/z values 179.07 (100%); 161.07 (20%);
 - Second fragmentation originated the m/z value 161.14 (100%);

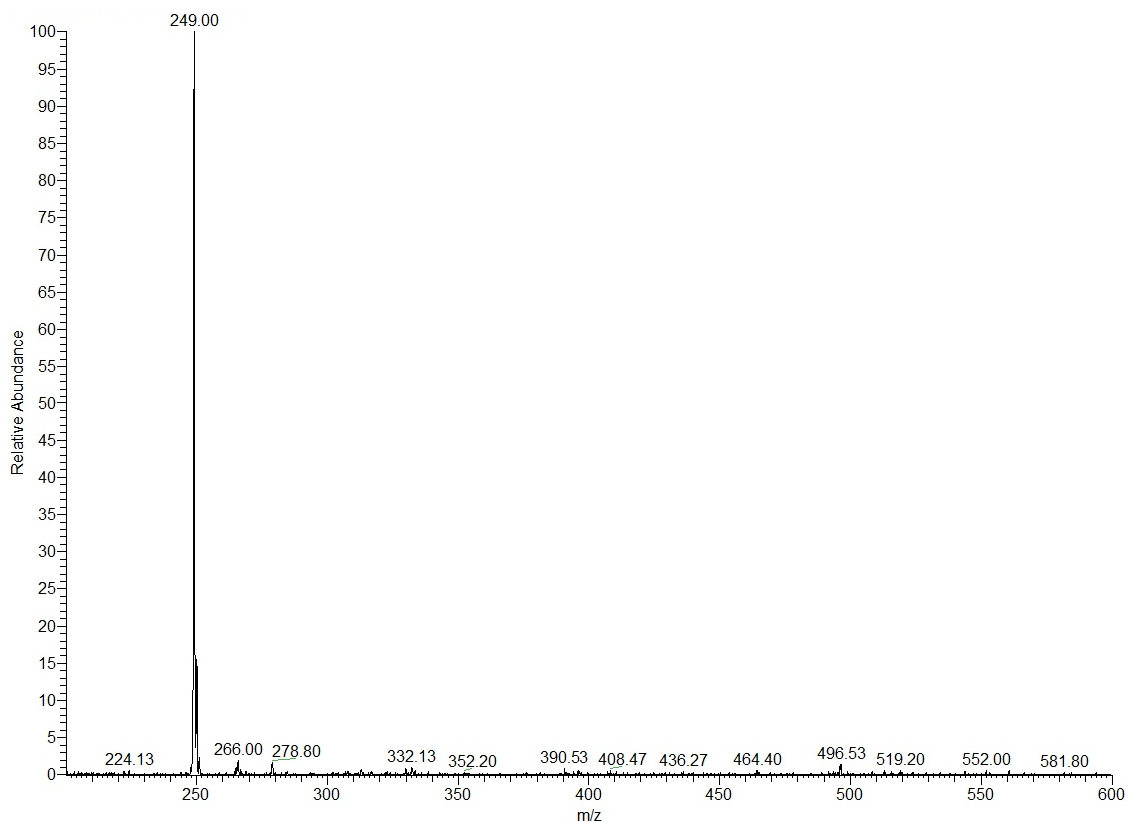


Figure S1. Mass spectrum of the parental compound, IMC.

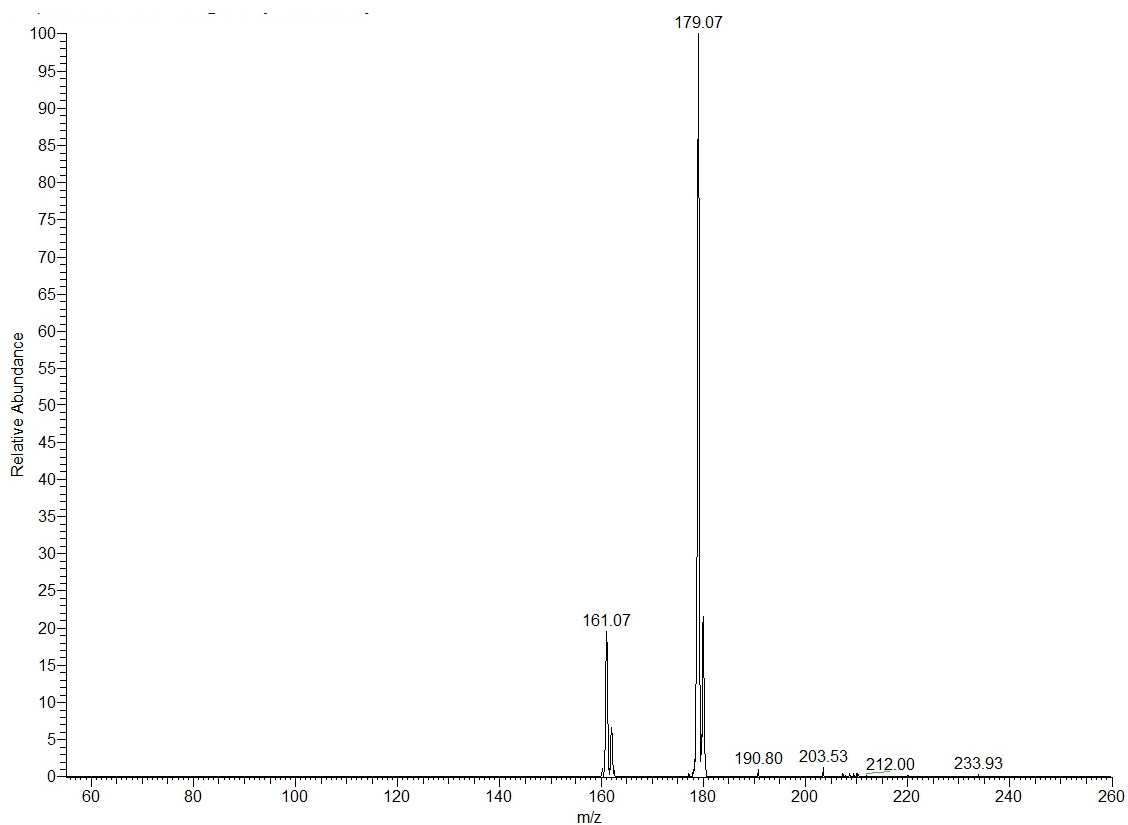


Figure S2. Mass spectrum of the first fragmentation of IMC.

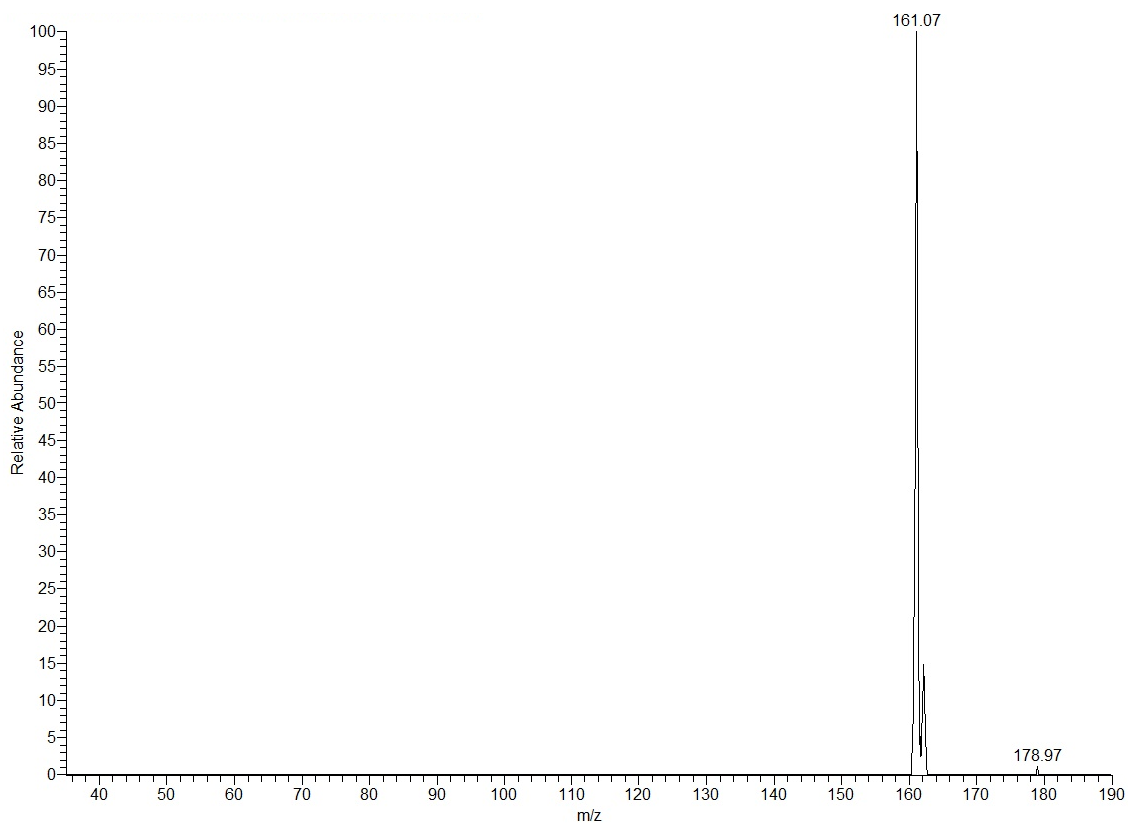


Figure S3. Mass spectrum of the second fragmentation of IMC.

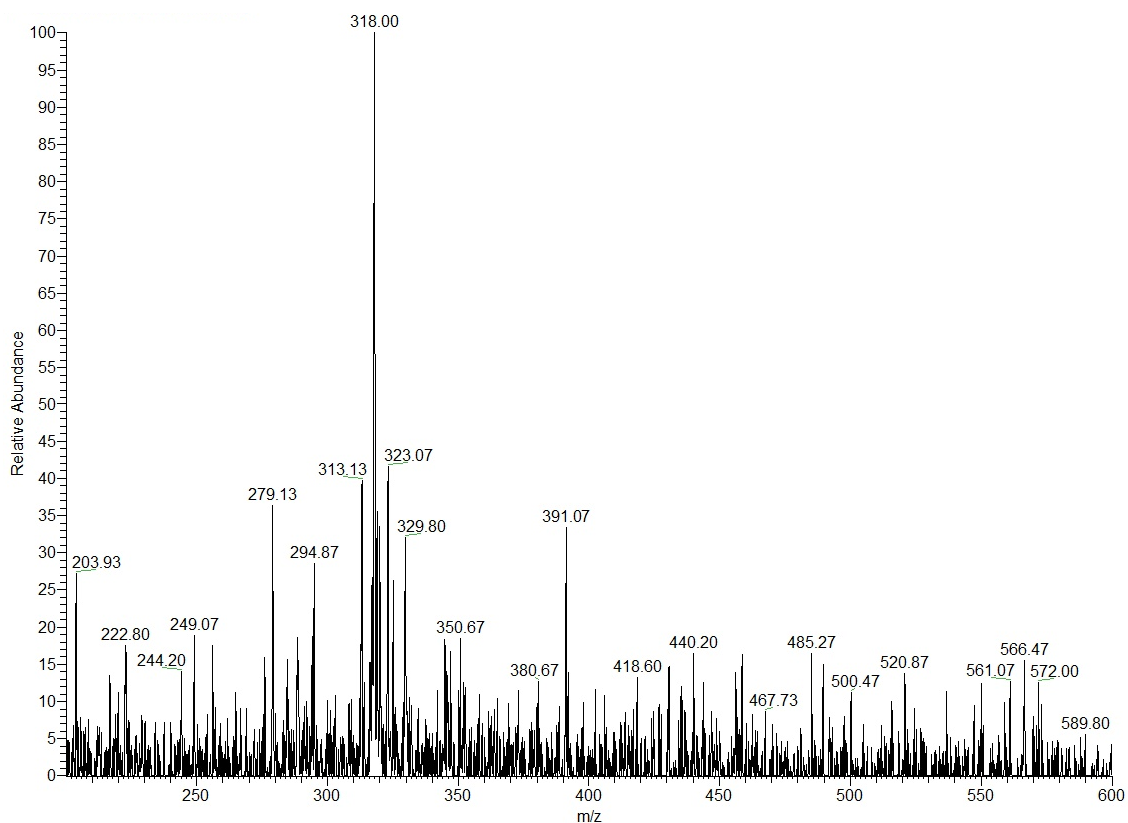


Figure S4. Mass spectrum of the dichlorinated DBP of IMC, Cl₂-IMC.

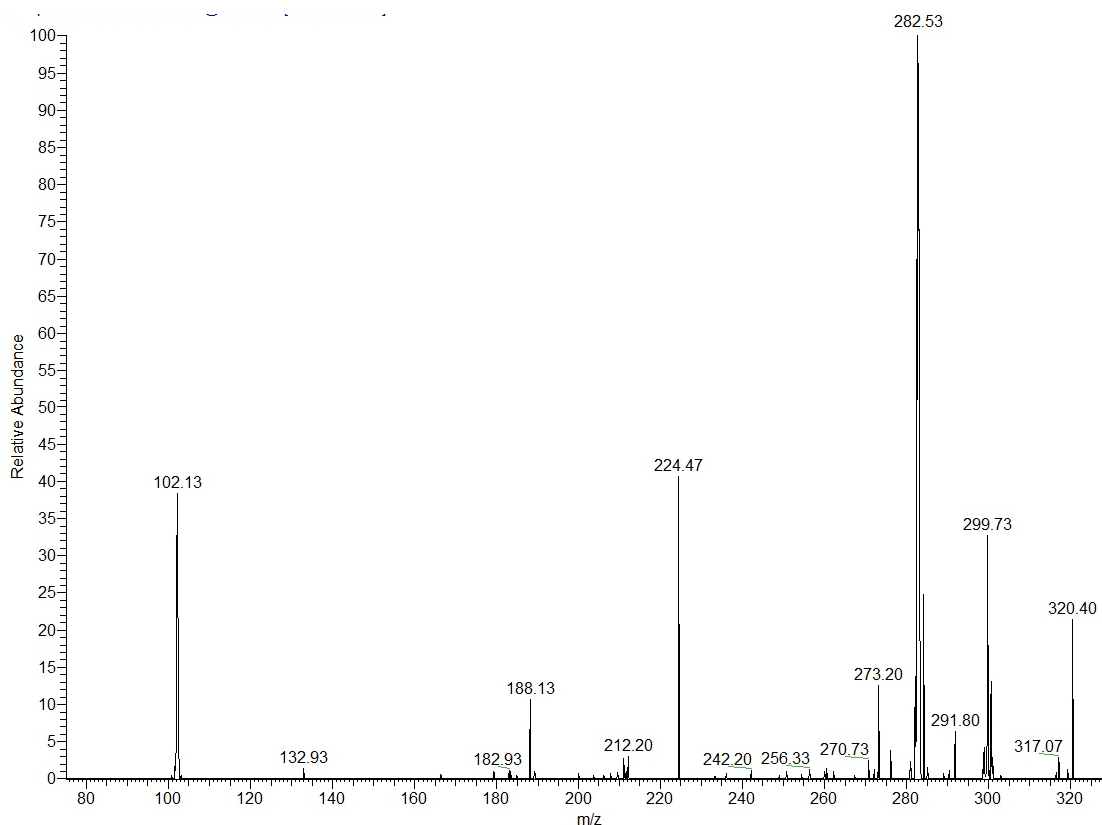


Figure S5. Mass spectrum of the first fragmentation of Cl₂-IMC.

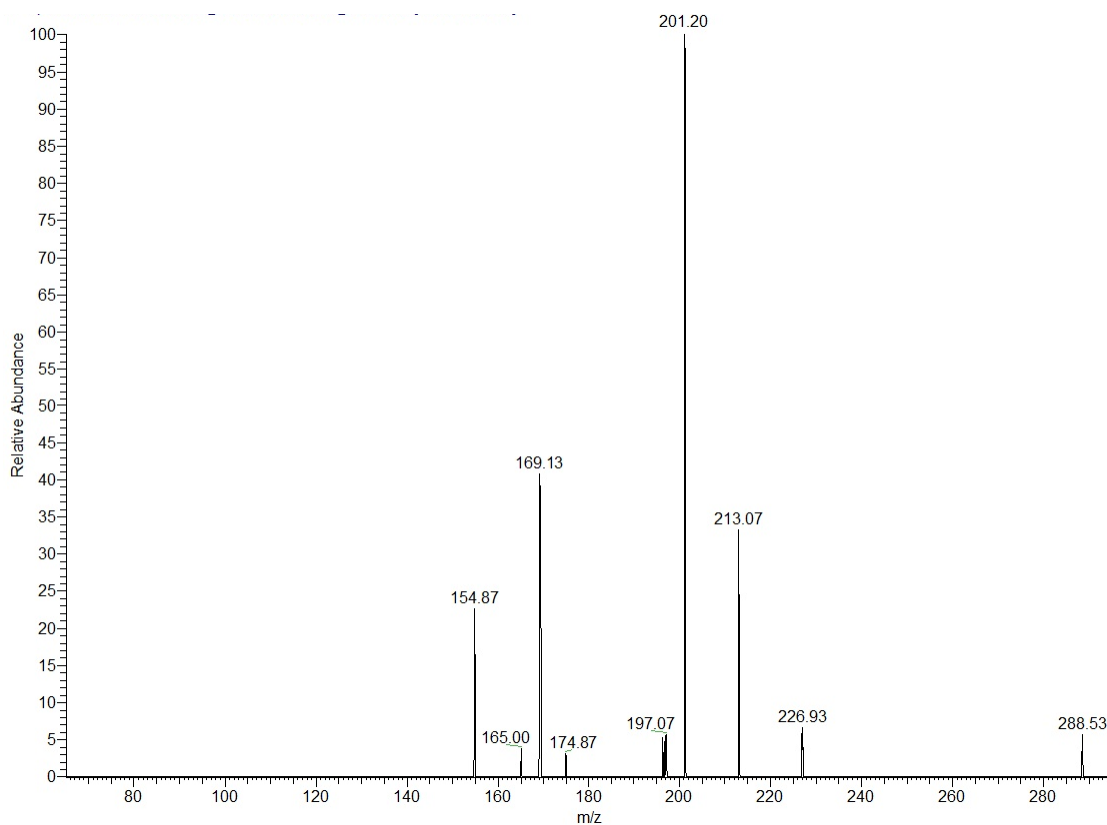


Figure S6. Mass spectrum of the second fragmentation of Cl₂-IMC.

2. Study of the influence of external variables on degradation

The detailed information on the experimental conditions of both Box-Behnken sets, are presented in [Tables S1 and S2](#).

Table S1. Experimental conditions for the first Box-Behnken design.

Experiment	[IMC] /mg/L	[NaOCl] /mg/L	pH	T /°C
1	0,5002	1,002	6	20
1b	0,5002	1,002	6	20
2	0,5002	3,002	6	20
2b	0,5002	3,002	6	20
3	0,5002	1,002	8	20
3b	0,5002	1,002	8	20
4	0,5002	3,002	8	20
4b	0,5002	3,002	8	20
5	0,5002	1,002	7	15
5b	0,5002	1,002	7	15
6	0,5002	3,002	7	15
6b	0,5002	3,002	7	15
7	0,5002	1,002	7	25
7b	0,5002	1,002	7	25
8	0,5002	3,002	7	25
8b	0,5002	3,002	7	25
9	0,5002	2,000	6	15
9b	0,5002	2,000	6	15
10	0,5002	2,000	8	15
10b	0,5002	2,000	8	15
11	0,5002	2,000	6	25
11b	0,5002	2,000	6	25
12	0,5002	2,000	8	25
12b	0,5002	2,000	8	25
Central a	0,5002	2,000	7	20
Central b	0,5002	2,000	7	20
Central c	0,5002	2,000	7	20

Table S2. Experimental condition for the second Box-Behnken design.

Experiment	[IMC] /mg/L	[NaOCl] /mg/L	[DOM] /mg/L	pH	T /°C
1	0,5002	1,002	0,1000	7	20
1b	0,5002	1,002	0,1000	7	20
2	0,5002	3,002	0,1000	7	20
2b	0,5002	3,002	0,1000	7	20
3	0,5002	1,002	5,0000	7	20
3b	0,5002	1,002	5,0000	7	20
4	0,5002	3,002	5,0000	7	20
4b	0,5002	3,002	5,0000	7	20
5	0,5002	1,002	2,5500	6	20
5b	0,5002	1,002	2,5500	6	20
6	0,5002	3,002	2,5500	6	20
6b	0,5002	3,002	2,5500	6	20
7	0,5002	1,002	2,5500	8	20
7b	0,5002	1,002	2,5500	8	20
8	0,5002	3,002	2,5500	8	20
8b	0,5002	3,002	2,5500	8	20
9	0,5002	1,002	2,5500	7	15
9b	0,5002	1,002	2,5500	7	15
10	0,5002	3,002	2,5500	7	15
10b	0,5002	3,002	2,5500	7	15
11	0,5002	1,002	2,5500	7	25
11b	0,5002	1,002	2,5500	7	25
12	0,5002	3,002	2,5500	7	25
12b	0,5002	3,002	2,5500	7	25
13	0,5002	2,000	0,1000	6	20
13b	0,5002	2,000	0,1000	6	20
14	0,5002	2,000	5,0000	6	20
14b	0,5002	2,000	5,0000	6	20
15	0,5002	2,000	0,1000	8	20
15b	0,5002	2,000	0,1000	8	20
16	0,5002	2,000	5,0000	8	20
16b	0,5002	2,000	5,0000	8	20
17	0,5002	2,000	0,1000	7	15
17b	0,5002	2,000	0,1000	7	15
18	0,5002	2,000	5,0000	7	15
18b	0,5002	2,000	5,0000	7	15
19	0,5002	2,000	0,1000	7	25
19b	0,5002	2,000	0,1000	7	25
20	0,5002	2,000	5,0000	7	25
20b	0,5002	2,000	5,0000	7	25
21	0,5002	2,000	2,5500	6	15
21b	0,5002	2,000	2,5500	6	15
22	0,5002	2,000	2,5500	8	15
22b	0,5002	2,000	2,5500	8	15

23	0,5002	2,000	2,5500	6	25
23b	0,5002	2,000	2,5500	6	25
24	0,5002	2,000	2,5500	8	25
24b	0,5002	2,000	2,5500	8	25
Central a	0,5002	2,000	2,5500	7	20
Central b	0,5002	2,000	2,5500	7	20
Central c	0,5002	2,000	2,5500	7	20

The ANOVA information of the first model (without DOM in solution), is presented in [Table S3](#). The variables evaluated were: NaOCl concentration; pH; water temperature; and their quadratic terms and interactions. Details on the statistical validation of the model are as follows:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the critical F -value (obtainable from the distribution tables of statistical literature) is 1.833;
- As presented in [Table S3](#), the experimental F -value of the model is 4.093, and therefore, significantly higher than the critical F -value just mentioned (1.833);
- Additionally, the P -value for the model is 0.006, and therefore quite lower than the significance level ($\alpha = 0.05$);

Since the experimental P -value (0.0060) is lower than the significance level (0.05) of the model, the obtained results are therefore proven to display statistical significance. Regarding the experimental F -value (4.093), it is quite higher than the critical value (1.833), which means that the relation between the variables and the obtained results is indeed also statistically significant. In conclusion, the data allows for the rejection of the null hypothesis (H_0), that states that there is no interaction or relation between the studied variables and that the differences found within the results are not statistically relevant, and accept therefore the alternative hypothesis (H_a), that defends the exact opposite, that is, the results and the interaction between the variables are statistically significant. As for the results, the most influential variables proved to be the water temperature (β -coefficient = -1.155 ± 0.299) and the interaction [NaOCl] x pH (β -coefficient = 3.673 ± 1.303). In parallel, these are also the factors with the highest F -values (14.895 for the water temperature, and 7.950 for the interaction [NaOCl] x pH). As expected, for the experimental P -values for these terms fall below the significance level of 5%.

Table S3. ANOVA data for the first model (without DOM in solution) on the study of influence of external variables on the degradation of IMC.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1,321E+03	9	146,733	4,093	0,006		
	Error	609,423	17	35,848				
	Adjusted total	1,930E+03	26	74,231				
Factors	Intercept	1,047E+04	1	1,047E+04	292,002	0	59,070	3,457
	[NaOCl] /mg/L (A)	1,600	1	1,600	4,464E-02	0,8352	0,316	1,497
	pH (B)	3,582	1	3,582	9,991E-02	0,7558	-0,473	1,497
	T /°C (C)	533,956	1	533,956	14,895	0,0013	-1,155	0,299
	AB	285,008	1	285,008	7,950	0,0118	3,673	1,303
	AC	4,190	1	4,190	0,117	0,7366	-0,445	1,303
	BC	53,561	1	53,561	1,494	0,2383	1,592	1,303
	AA	22,645	1	22,645	0,632	0,4377	1,232	1,550
	BB	311,011	1	311,011	8,676	0,009	-4,567	1,550
	CC	1,377	1	1,377	3,840E-02	0,8470	0,304	1,550
Model check	Main	539,138	3	179,713				
	Int	342,759	3	114,253	3,187	0,0504		
	Int + Sqr	438,696	3	146,232	4,079	0,0236		
	Squ	438,696	3	146,232	4,079	0,0236		
	Error	609,423	17	35,848				
Lack of fit	Lack of fit	608,547	3	202,849	3,243E+03	0		
	Pure error	0,876	14	6,254E-02				
	Total error	609,423	17	35,848				

In regards to the second model (with DOM included in solution), the ANOVA data is presented in Table S4. The variables studied were: NaOCl concentration; pH; DOM concentration; water temperature; and the corresponding quadratic terms and interactions. Statistical validation of the model was achieved as follows:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the critical F -value (obtainable from the distribution tables of statistical literature) is 1.761;
- As presented below in Table S4, the experimental F -value of the model is 2.685, and therefore, higher than the critical F -value just mentioned (1.833);
- The P -value for the model is 0.0086, and therefore quite lower than the significance level (0.05);

As was explained above, the experimental P -value (0.0086) is lower than the significance level of the model (0.05), therefore, the results exhibit statistical significance. The experimental F -value (2.685), is higher than the critical value (1.833), meaning that the interaction between the variables and the relation between the obtained results, are also statistically significant. Once again, data allows for the safe rejection of the null hypothesis (H_0) in favour of the alternative hypothesis (H_a). The most influential variables on degradation of IMC, in the second model, were [DOM] (β -coefficient = 1.803 ± 0.358), and to less extent, the pH (β -coefficient = -1.235 ± 0.877) and the interaction [NaOCl] x pH (β -coefficient = -1.270 ± 0.729). As in the case of the first model, these variables were also the ones with the highest F -values (25.392 for DOM concentration, 1.985 for the pH and 3.036 for the interaction [NaOCl] x pH). Their experimental P -values were also found to be below the significance level of 5%.

Table S4. ANOVA data for the second model (with DOM in solution) on the study of influence of external variables on the degradation of IMC.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	693,634	14	49,545	2,685	0,0086		
	Error	664,265	36	18,452				
	Adjusted total	1,358E+03	50	27,158				
Factors	Intercept	7,442E+03	1	7,442E+03	403,327	0	49,807	2,480
	[NaOCl] /mg/L (A)	2,059	1	2,059	0,112	0,7403	-0,293	0,877
	[DOM] /mg/L (B)	468,520	1	468,520	25,392	0	1,803	0,358
	pH (C)	36,630	1	36,630	1,985	0,1674	-1,235	0,877
	T /°C (D)	6,510	1	6,510	0,353	0,5562	-0,104	0,175
	AB	6,160	1	6,160	0,334	0,5670	0,421	0,729
	AC	56,021	1	56,021	3,036	0,0900	-1,270	0,729
	AD	0,157	1	0,157	8,498E-03	0,9271	6,720E-02	0,729
	BC	14,072	1	14,072	0,763	0,3883	-0,637	0,729
	BD	1,288	1	1,288	6,980E-02	0,7931	-0,193	0,729
	CD	1,488	1	1,488	8,063E-02	0,7781	-0,207	0,729
	AA	7,718	1	7,718	0,418	0,5219	0,491	0,759
	BB	4,805	1	4,805	0,260	0,6129	0,387	0,759
	CC	24,455	1	24,455	1,325	0,2572	0,874	0,759
	DD	16,551	1	16,551	0,897	0,3499	-0,719	0,759
Model check	Main	513,720	4	128,430				
	Int	79,186	6	13,198	0,715	0,6397		
	Int + Sqr	100,728	4	25,182	1,365	0,2654		
	Squ	100,728	4	25,182	1,365	0,2654		
	Error	664,265	36	18,452				
Lack of fit	Lack of fit	661,333	10	66,133	586,574	0		
	Pure error	2,931	26	0,113				
	Total error	664,265	36	18,452				

2.2. The UV filter hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB)

This study was entirely carried out and fulfilled by the author, Albano Joel Moreira Santos, with supervision, guidance and revision being provided by Professor Dr. Joaquim Carlos Gomes Esteves da Silva.

This following manuscript, represents the submitted version of the article, currently in review and awaiting publication.

Fate and behaviour of the UV filter and emerging pollutant hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB) in aqueous solution

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Abstract

The stability of the ultraviolet (UV) filter hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB) is investigated, and its photo-induced and disinfection-induced degradation potential are assessed in aqueous solution. High performance liquid chromatography coupled with ultra-violet, diode array detection (HPLC-UV-DAD), was used to study the degradation reactions and determine kinetic features. Liquid chromatography coupled with diode array detection and mass spectrometry (LC-DAD-MS), was used to determine any potential by-products detected in the degradation reactions. The evaluation of external aquatically-relevant variables on degradation, such as pH, active chlorine concentration, temperature or dissolved organic matter (DOM) concentration, was carried out by means of a Box-Behnken experimental design formulation with factorial analysis. Results have shown that DHHB degrades quite rapidly in common drinking water disinfection conditions, displaying a kinetic rate constant of $0.0060 \pm 0.0002 \text{ s}^{-1}$ and a half-life period of merely 116 ± 4 seconds. As far as the non-volatile disinfection by-products (DBPs) are concerned, only the mono and dichlorinated forms of DHHB were detected in the degradation reactions. Regarding the external influences on DHHB degradation, the absence or presence of DOM did not alter the trends that were found: degradation of DHHB is more significant at lower pH values and higher levels of active chlorine in solution. Chlorinated DBPs have also been found to predominate under higher pH values and lower levels of chlorine, whereas they were found to be unstable and further degrade, quite likely into more volatile compounds, when in lower pH and higher chlorine concentrations. As for the photo-degradation studies, DHHB was found to be extraordinarily stable, with only about 15% degradation rate detected during an artificial irradiation period of 6 hours, and an UV absorption capacity decay of about the same amount during that period.

Keywords

UV filters; DHHB; Uvinul A Plus; active chlorine; disinfection by-products; DBPs;

1. Introduction

Most UV filters are organic compounds that absorb UVA (320-400 nm) or UVB (290-320) radiation, therefore protecting human health and skin against the effects of exposure to the nefarious radiation. Subsequently, these compounds represent the primary constituents of sunscreens and other personal care products and/or cosmetics [1, 2].

Up to 2007, there were 55 UV filters approved and regulated worldwide, the overwhelming majority of which were of synthetic organic nature along with two inorganic compounds, zinc oxide and titanium dioxide [2]. Focusing on the vastly more popular and numerous, the organic UV filters display the most common structural features of priority organic pollutants (POPs), that is, one or two aromatic moieties with unsaturated side-chains, promoting efficient electronic delocalization. Additionally, these compounds also display significant persistence and accumulation within the environment and food chain [1-4].

Along with the concerns regarding the serious effects of UV radiation on human skin and health, which naturally leads to the increase of the use or application of sunscreen products, there are also worries focused on the prominent lack of knowledge about the fate and behaviour of most of the UV filters when in the aquatic environments [3, 5, 6]. Their paths of inclusion into superficial water courses, have been addressed in detail in a comprehensive review by Giokas [3], underlining recreational activities such as bathing (in showers, swimming pools, rivers and lakes), actions such as rubbing off (on towels and clothing in general, with these being laundered and introducing the compounds into the municipal water bodies), and physiological responses such as excretion, as the main factors. Wastewater treatment, sewage or landfill overflows, stand as important paths of inclusion into the environment, as well [3, 6].

Given the nature of these compounds and the products in which they are mostly applied (sunscreens), their levels present the highest values during the summer and in locations of typical, popular and intense recreational aquatic activities (coastal ocean waters, swimming pools, rivers and lakes) [5]. However, despite their purpose and ability to protect against UV radiation, these compounds are known to experience significant degradation in aqueous solution, both photo-induced or disinfection-induced, yielding the production of degradation by-products which can also present potential eco-toxicological profiles [4-6]. Despite the use of sunscreens being mostly

seasonal and the levels of UV filters in the aquatic environmental compartments increasing, therefore, during the Summer, these compounds display significant persistent and accumulative character [5, 7]. In addition, the parental compounds are also linked to several potential toxicological effects, themselves, predominantly hormonal and anti-hormonal, as was addressed by Díaz-Cruz [4], with many of them presenting multiple toxicological profiles.

Presently, there are merely a few studies dealing with the degradation reactions of UV filters in aqueous solution. Most or all of these publications are only focused on one of the two degradation scenarios, namely the disinfection-induced degradation, leaving out the photo-degradation context. Sakkas published the only research dealing simultaneously with disinfection-induced and photo-induced degradation, but he focused on the UV filter 2-ethylhexyl-4-(dimethylamino)-benzoate (EHPABA) in distilled, ocean and swimming pool waters [8]. Negreira studied the filters 2-ethylhexyl salicylate (ES), 2-ethylhexyl-4-(dimethylamino)-benzoate (EHPABA), and benzophenone-3 (BP-3) in chlorinated water samples [9]. Nakajima investigated the degradation reactions of the UV filters 2-ethylhexyl-4-methoxycinnamate (EMC) and EHPABA with active chlorine in aqueous solution [10]. Our research group studied the filters EMC and 4-*tert*-butyl-4'-methoxydibenzoylmethane (BDM) also with active chlorine in aqueous solution [11]. Grbović approached merely the mass spectrometry identification of DBPs of the filter 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB) in chlorinated aqueous solutions, but without addressing the degradation reactions of the filter [12]. Negreira also investigated the reactivity of benzophenone-4 (BP4) towards active chlorine [13]. And finally, Duirk focused on the kinetic characteristics of the degradation reaction of benzophenone-3 (BP3) and benzophenone-8 (BP8) with active chlorine [14].

As was pointed out before, most of the aforementioned studies simply deal with either the stability of the compounds in aqueous solution or, in one specific case, after UV-radiation exposure [7], but seldom with both degradation scenarios accounted. Therefore, the purpose of the present work is directed at the two weak points of UV filter research: the vast majority of compounds being still unknown, and, in the case of studied compounds, their incomplete fate and behaviour assessment. Subsequently, this study is therefore focused on the determination of the fate and behaviour of the UV filter hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB) in aqueous solution and experimental conditions mimicking, as close as possible, superficial aquatic contexts relevant to human activity, such as swimming pool water or

municipal/drinking water. It should also be highlighted that the studies will focus on a compound which is completely unknown and unapproached in either of the degradation scenarios presented.

The molecular structure of DHHB is presented in [Figure 1](#) while its physical-chemical properties are included in [Table 1](#). More objectively, this work aims to determine kinetic degradation rates of both photo-induced and disinfection-induced degradation reactions, evaluate the effects of photo-degradation on the compound's UV absorption features, successfully identify potential non-volatile degradation by-products and determine the effect of common external variables, such as active chlorine levels, pH, water temperature and dissolved organic matter (DOM) levels, on degradation rates. In order to accomplish these goals, the degradation reactions will be followed by high-performance liquid chromatography coupled with ultraviolet, diode-array detection (HPLC-UV-DAD), with aliquots of the reaction mixtures being directly injected into the system and in real time. Photo-irradiation experiments were performed with an artificial solar radiation simulator with air mass filters and by using a liquid light guide. Potential by-products being detected in both the degradation reactions, were studied and determined by a highly sensible liquid chromatography system coupled with diode-array detection and mass spectrometry (LC-DAD-MS), with a quadrupole ion trap, for more accurate and precise identification of compounds. To promote the successful determination of all by-products, and to emphasize reliability on the detection process, a solid phase extraction (SPE) protocol was always implemented, prior to the analysis in the LC-DAD-MS system, with the extracts being also directly injected into the system after the respective recovery. Influence of external variables on degradation, was evaluated by means of an experimental design formulation, allowing the detection of degradation trends in the reactions by statistical analysis.

2. Materials and methods

2.1. Reagents

The UV filter DHHB (CAS registry number 302776-68-7, molecular formula $C_{24}H_{31}NO_4$) was purchased as an analytical grade standard from Sigma-Aldrich. A standard reagent grade aqueous sodium hypochloride (NaOCl) solution, with 3.5% active chlorine labelled content, was purchased from VWR Chemicals. From this solution, a

stock solution of 432 mg/L in active chlorine content was prepared and permanently stored at 4°C and removed only upon use. Active chlorine content was periodically evaluated by means of the standard iodometric titration method. Deionized water (conductivity < 0.1 $\mu\text{S}\cdot\text{cm}^{-1}$) was used in all experiments. The pH of the studied experimental solutions was always adjusted with the addition of conventional 0.1 mol/L standard solutions of either hydrochloric acid (HCl) or sodium hydroxide (NaOH). Pre-concentration of the aqueous reaction mixtures, necessary for the LC-MS-DAD analysis and identification of DBPs, was carried out by solid-phase extraction (SPE) using 6 mL, 500 mg hydrophilic-lipophilic balance (HLB) cartridges from Waters Corporation, Milford, MA. All organic solvents used for pre-concentration or chromatographic purposes, were of liquid chromatographic grade and purchased from Merck. Dissolved organic matter (DOM) was obtained from pinewood soil [15-17].

2.2. Chromatographic conditions

2.2.1. HPLC-UV-DAD

High performance liquid chromatography coupled with ultraviolet diode array detection (HPLC-UV-DAD), was used to follow the DHHB degradation reactions along with time.

The HPLC-UV-DAD system was composed of an isocratic pump (Hewlett–Packard 1100 Series, Böblingen, Germany), a manual sample injection valve with a 20 μL loop (Rheodyne 7725i, Rohnert Park, USA), a silica-based C18 reversed phase column [Dionex Acclaim™ 120 C18, particle size 3 μm , pore diameter 175 Å (4.6 × 150 mm), Thermo Fisher Scientific, Sunnyvale, CA, USA] and a photodiode array detector (UV 6000LP with a 50 mm LighPipe flow cell, Thermo Fisher Scientific, Sunnyvale, CA, USA). The mobile phase used to follow the degradation reactions, was a mixture of liquid chromatography grade acetonitrile and deionized water (80:20, v:v), at a constant 0.60 mL/min flow rate. Before use, mobile phases were always filtered by pressure through a borosilicate glass funnel (pore size 4-8 microns). Absorbances were monitored and recorded at a total scan mode (200-600 nm), and the entire system controlled with the Xcalibur 1.4 SR software.

2.2.2. LC-DAD-MS

Liquid Chromatography coupled with Mass Spectrometry and Diode Array Detection (LC-MS-DAD) was used to study the formation of by-products of DHHB degradation.

The LC-MS-DAD system was composed of a HPLC pump (Finnigan Surveyor LC Pump Plus), an auto-sampler (Finnigan Surveyor Auto-sampler Plus) and a photodiode array detector equipped with a LightPipe flow cell (Finnigan Surveyor PDA Plus Detector) (all instrumentation was purchased from Thermo Electron Corporation, Waltham, USA), coupled with the same reverse phase silica-based column [Dionex Acclaim™ 120 C18, particle size 3 µm, pore diameter 175 Å (4.6 × 150 mm), Thermo Fisher Scientific, Sunnyvale, CA, USA]. The mass spectrometer was a Finnigan LCQ Deca XP Max (Thermo Electron Corporation, Waltham, USA) coupled to the LC system, equipped with an electrospray interface as ionization source and a quadrupole ion trap for MSⁿ experiments, and it was operated in positive ion mode between m/z = 150 and 1000 with the following conditions: spray voltage, 5.0 kV; capillary voltage, 15 V; and capillary temperature, 300°C. The device was programmed to firstly perform a full mass scan and then a fragmentation analysis (MS/MS) of the most intense ion. The mobile phase was similar to the one used with the HPLC system, with acetonitrile and deionized water but this time including 0.1% of formic acid (HCOOH) (80:19.9:0.1, v:v), in order to promote the ionization of the compounds of interest, and it was pumped through the system at the same flow rate of 0.60 mL/min. Similarly to the procedure used with the HPLC system, mobile phases were always filtered prior to use, with a borosilicate glass funnel (pore size 4-8 microns). The system was also controlled using the Xcalibur 1.4 SR software.

2.2.3. Performance of the analytical method

The analytical method was evaluated, optimized and validated based on the standard ICH guidelines [18]. Calibration was carried out by analysing 9 standard solutions ranging between 0.02500 mg/L (6.290×10^{-8} mol/L) and 25.00 mg/L (6.300×10^{-5} mol/L). Three trials were done for each solution. Correlation coefficients (R^2) were all determined to be higher than 0.998.

2.3. Disinfection-induced degradation studies

2.3.1. Determination of reaction kinetics

An experimental design was assembled, focusing on being as simple and objective as possible, using deionized water as the experimental medium, at neutral pH, room temperature, in the dark and under constant stirring.

A stock solution of DHHB was first prepared by dissolving 25 mg of standard compound in 100.00 mL of absolute ethanol (final concentration being 250.00 mg/L (or 6.289×10^{-4} mol/L)). All the subsequent experiments were prepared by using this stock solution as the source of DHHB.

Experimental mixtures were prepared and carried out in 250 mL ordinary borosilicare glass vessels, containing 1.000 mg/L (2.516×10^{-6} mol/L) of DHHB and 5.002 mg/L (6.716×10^{-5} mol/L) of NaOCl in 100 mL of deionized water, ensuring that the variance in the levels of active chlorine along with time remained negligible, in theory. An initial aliquot of the solution containing solely the DHHB, was always analyzed by direct injection in the HPLC-UV-DAD system, and prior to the addition of NaOCl, in order to ensure a chromatographic record of the initial amount of the compound of interest. Subsequently, NaOCl was included, pH verified, monitored and regulated if necessary ($\text{pH} = 6.4 \pm 0.5$), the water temperature recorded and also monitored ($T = 20.7 \pm 0.1^\circ\text{C}$) and the reaction timer initiated. One aliquot of 1000 μL was taken from the reaction mixture at every 20 seconds of reaction, during a 4-minute total reaction time-frame. Their residual active chlorine in solution was quenched with the addition of 10 μL of a standard ascorbic acid (AA) solution of approximately 65 g/L, and the samples were immediately stored at 4°C until the very moment of analysis by direct injection in the HPLC-UV-DAD system. This procedure allowed the verification and determination of the degradation grade of DHHB along with time.

2.3.2. Identification of non-volatile DBPs

This portion of the work was focused on the determination of non-volatile DBPs as a result of the disinfection-induced degradation reaction. The experimental conditions were very much analogous to those described in the previous section, but in this case, the solution was left undisturbed for 10 minutes, a period after which the entire reaction mixture was pre-concentrated by SPE [using 6 mL, 500 mg hydrophilic-lipophilic balance (HLB) cartridges from Waters Corporation, Milford, MA]. Before pre-concentration, the cartridges were conditioned with 3 mL of methanol followed by 3 mL of deionized water. The entire solution was then made to pass through the cartridge with the help of a peristaltic pump (Minipuls® 2 from Gilson, Inc., Middleton, WI, USA). The analytes of interest adsorbed in the cartridge were subsequently extracted with 3 mL of dichloromethane, and the organic solvent was then evaporated with a careful stream of nitrogen gas, with the final residue being diluted in 500 μL of methanol and analysed immediately after by direct injection in the LC-DAD-MS system.

2.3.3. Study of influence of external variables on degradation

This part of the work was ensured by using an experimental design formulation approach and the subsequent statistical analysis (ANOVA) of the results and its response surface calculations. The process was enabled by The Unscrambler® v9.2 (CAMO Process AS, Oslo, Norway) software program.

The purpose of this section of the work, was to investigate the extent of influence that prominent, common external variables [such as the pH, water temperature, active chlorine concentration and dissolved organic matter (DOM) levels] have on the fate and behaviour of organic pollutants in the aquatic, environmental compartments. With this goal in mind, two Box-Behnken designs were created, one with and another without DOM in solution. The details and design formulation features of the designs, are presented in [Figure 2](#). Additional and more detailed information on the experimental conditions of the experiments, is presented in the supplementary material. The experimental variables, along with the corresponding conditions, were defined based on knowledge from previous studies [\[5, 8-11\]](#). In other words, there was the need to establish experimental scenarios as close as possible to actual superficial waters bodies, such as swimming pool waters, but one that could also enable the accurate perception of the degradation reactions with the HPLC analysis.

2.4. Photo-degradation studies in aqueous solution

The photo-degradation experiments were carried out by artificial solar irradiation, using a solar radiation simulator (Newport Oriel 96000 150 W) with Air Mass Filters 1.5 Global 81094 and a liquid light guide, the tip of which was positioned about 1 cm above the aqueous solutions.

As in previous sections, reactions were performed in ordinary borosilicate glass beakers containing 100 mL of deionized water, spiked with DHHB to generate a concentration of 1.000 mg/L (2.516×10^{-6} mol/L). In parallel with the irradiated solutions, another equivalent solution was always kept in the dark and ambient temperature, also analysed at each step as a control. Irradiation experiments lasted for a total time-frame of six hours, with an aliquot of each solution (irradiated and control) being removed at each hour and analysed by direct injection in the HPLC-UV-DAD system. Prior to the analysis in the HPLC-UV-DAD system, the samples were always analysed in the UV-Vis spectrophotometer. Thus, it was possible to determine the stability of DHHB in

aqueous solution while being irradiated, and also ascertain the possible changes in the compound's UV spectrum profiles.

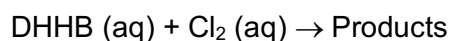
3. Results and discussion

3.1. Disinfection-induced degradation

3.1.1. Determination of reaction kinetics

The only existing study focused on DHHB, was published in 2013 [12], and it dealt solely with the mass spectrometry (MS) determination of its by-products without approaching the degradation reaction itself. Subsequently, this work intended to fill that gap and investigate this experimental scenario, by determining the compound's degradation profile when in contact with active chlorine, as well as confirming the non-volatile DBPs originated in the process. It is also important to underline, once again, that the purpose of the disinfection-induced degradation study, was to reflect and present an experimental scenario as close as possible to the one usually found in municipal waters, namely in what regards the active chlorine concentrations in aqueous solution [19, 25, 26].

As Deborde and Von Gunten underline [19], oxidation reactions between organic or inorganic compounds and active chlorine, usually follow a total second-order kinetic degradation profile, being first-order for both the compound and active chlorine. Therefore, regarding the reaction between DHHB and the total amount of active chlorine in solution ($[Cl_2]_t = [HOCl] + [ClO^-]$), we have:



And so, the corresponding kinetic law will be:

$$v = -(\Delta[DHHB]/\Delta t) = k_{app}[DHHB][Cl_2]_t$$

Where $[DHHB]$ is the concentration of the UV filter, $[Cl_2]_t$ is the total concentration of active chlorine species in solution ($HOCl + ClO^-$), the presence or predominance of which is pH-dependent, and k_{app} is the apparent global second-order rate constant. Under pseudo-first order conditions, the kinetic law can be simplified:

$$-(\Delta[DHHB]/\Delta t) = k_{obs}[DHHB]$$

Where k_{obs} is the observed pseudo first-order rate constant of the studied reaction ($k_{\text{obs}} = k_{\text{app}}[\text{Cl}_2]_t$).

The focus of this part of the study, was to determine the observed pseudo first-order rate constant of the degradation reaction and ascertain the half-life period of DHHB in aqueous solution with exposure to active chlorine. Figure 3 presents the exponential plot of the concentration of DHHB as a function of reaction time. According to the experimental results:

$$[\text{DHHB}]/[\text{DHHB}]_0 = 1.068645e^{-0.005956t}$$

From which it was possible to determine, by linear regression, the observed pseudo first-order rate constant ($k_{\text{obs}} = 0.0060 \pm 0.0002 \text{ s}^{-1}$) and the specific half-life period of the compound ($t_{1/2} = 116.38 \pm 3.71 \text{ s}$). The data confirms the fact that DHHB is highly reactive in a context of exposure to active chlorine, reaching half of its initial concentration slightly under two minutes, and achieving around 74% degradation rate at the end of the four minutes of reaction time-frame. There are currently no other studies dealing with the determination of the kinetic features of the DHHB reactivity with exposure to active chlorine in aqueous solution, in order to offer a point of reference or analogy on possible similarities or differences as to the fate and behaviour of this compound in a context analogous to the superficial, aquatic, environmental compartments. However, other works have presented similar studies, albeit, with different compounds: such are the cases of EMC and BDM, studied by this same research group [11]; BP4, studied by Negreira and collaborators [13]; and BP3 and BP8, approached by Duirk and colleagues [14]. According to Negreira [13], BP4 half-life periods ($t_{1/2}$) varied between 3.41 (pH = 6.3), 0.78 (pH = 7.3) and 0.45 (pH = 8.1) minutes, in ultrapure water, and between 5.25 (pH = 6.3), 1.86 (pH = 7.3) and 0.98 (pH = 8.1) minutes, in ordinary municipal water. This shows us that reactivity of BP4 seems to increase with the increase of pH, which indicates higher reactivity of the filter with hypochloride anion (ClO^-), that predominates at alkaline conditions [19]. However, in the context of municipal water disinfection, such context is irrelevant, since drinking water disinfection occurs at slightly acidic conditions due to the significantly more efficient role of hypochlorous acid (HClO) as a disinfecting agent, and which predominates at acidic to neutral pH [19]. Still, at slightly acidic or neutral pH, BP4 displays lower reactivity than DHHB, while still being quite reactive itself. As for Duirk's results [14], the observed first-order rate constants (k_{obs}) obtained, were all below one tenth of the value determined in this study for DHHB, at pH = 6-7 and around the same

levels of active chlorine in solution, which also indicates much lower reactivity than DHHB, under drinking water disinfecting conditions. As for the results obtained by this research group for EMC and BDM, the former presented a half-life period of 73 minutes, whilst the latter had a half-life period of 119 minutes, under similar experimental conditions. Both these compounds have proven to be much more stable towards disinfection-induced degradation.

3.1.2. Identification of non-volatile DBPs

The LC-DAD-MS chromatogram displaying the determined mono and dichlorinated DBPs of DHHB, along with the parental compound and their respective MS spectra, is provided in [Figure 4](#). Additional information, namely the proposed mechanisms and molecular structures of the identified DBPs as well as all the mass spectra and fragmentations carried out for each compound, are included in the supplementary material ([Figures S1-S4 and S5-S11](#), respectively). Retention times and most prominent or abundant fragments are included in [Table 2](#).

Through the analysis of the data from [Table 2](#), it was possible to clearly identify two DBPs of DHHB, the mono and dichlorinated forms of the compound, also indicated in [Figure 4](#). The peak at 9.17 minutes was already known to belong to the parental compound DHHB, and the LC-MS-DAD analysis confirmed that fact. The MS analysis of the peak yielded the molecular ion 398 m/z, which is in accordance with the molecular mass of the filter (see [Table S3](#) in the supplementary material). Fragmentation of this peak, gave origin to the molecular ions 149, 233 and 296 m/z as the most abundant fragments. The two remaining peaks were assumed to be potential DBPs of DHHB, since, in parallel to their significance and intensity in the chromatogram, their area always varied as a function of variable DHHB or NaOCl concentrations in solution, throughout the studies. The peak at retention time 4.92 minutes exhibited a molecular ion of 466 m/z, consistent with the molecular mass of the dichlorinated form of DHHB. The fragmentation of this peak led to the molecular ions 149, 233, and 364 m/z as the most predominant fragments. As far as the peak at retention time 13.37 minutes is concerned, it displayed the molecular ion 432 m/z, consistent with the molecular mass of the mono-chlorinated form of DHHB. Its fragmentation yielded the ions 330, 302 and 348 m/z as the most intense fragments. [Figure 5](#) presents the UV absorption spectra of DHHB and both the identified DBPs, for comparison. Regarding the dichlorinated DBP, as [Figure 5](#) shows, its UV absorption spectrum is considerably different from the remaining two compounds (which were

able to retain a somewhat similar UV absorption profile), since its absorption band shifts towards 260 nm and with an absorption capacity below 40% of that originally displayed by DHHB. Grbović reported two apparently different monochlorinated species of DHHB, which were not found in this study, and in any of the experimental scenarios applied. The author also did not report the UV absorption spectra of the dichlorinated DBP, so we cannot comment of possible differences or similarities.

Referring once again to Deborde's and Von Gunten's comprehensive review work on the chlorination of organic and inorganic compounds [19], active chlorine will most likely attack aromatic rings by electrophilic substitution on the benzenic ring, particularly in *ortho* or *para* positions to important functional groups, such the hydroxyl and the tertiary amine groups present in one of DHHB's rings, due to their *ortho* and *para* activating role. Subsequently, given the strong *ortho-para* activating role of the -OH and -NR₂ groups, the chlorination reaction of DHHB will most likely result in the successive electrophilic substitution of chlorine into sites 1 (*ortho* position) and 2 (*para* position) marked in red on Figure 6. Taking into consideration the work of Deborde and Von Gunten [19], most noteworthy in this case, the fact that the electrophilic substitution of chlorine into the *ortho* position towards the hydroxyl group displays a rate constant 4 times higher than the one reported for the substitution in *para* position, it is safe to assume that the only monochlorinated DBP determined in this work (peak at 13.37 minutes in Figure 4), will mostly be the *ortho*-monochlorinated DHHB, presented in Figure 6. The dichlorinated form of DHHB (peak at 4.92 minutes in Figure 4 and structure presented in Figure 6) would have been originated from the successive substitution of chlorine into the *para* position of the already *ortho*-monochlorinated DHHB.

3.1.3. Study of the influence of external variables on degradation

As mentioned in section 2.3.3, two experimental designs were created, with and without DOM in solution, in order to ascertain the degree of influence of significant environmental variables on degradation of DHHB in aqueous solution. Detailed information about the analysis of variance (ANOVA) made on the experimental results, is presented in the supplementary material provided (see Tables S3-S6). It is also important to underline again that two non-design variables were measured or studied, the chlorination and effective degradation percentages, in order to ascertain the correct effect of the design variables.

First and foremost, it is important to underline that the models for the chlorination and effective degradation percentages from Batch A and B, are all statistically valid and significant, as the analysis present in section 2 of the supplementary material clearly indicates. As far as the results are concerned, data indicates that it is mostly the pH that influences chlorination and effective degradation in Batch A, with chlorine concentration right below it. As for temperature, it has proven to be insignificant in both chlorination and effective degradation of DHHB. Figures S12-S15, present in the supplementary material, show the response surfaces obtained for chlorination and effective degradation, respectively, combining the two most significant variables, pH and NaOCl concentration. The analysis of Figure S12 shows that the highest concentration of non-volatile chlorinated by-products was found at higher levels of pH and lower levels of active chlorine in solution, whilst the lowest amount of those compounds was found at the highest concentration of chlorine and lowest pH values. As far as the loss in DHHB concentration (see Figure S13), the trend is exactly the opposite, that is, degradation of the compound is achieved at higher levels of chlorine and at lower pH levels. Nakajima [10], also reported that the degradation rates of another UV filter, 2-ethylhexyl-4-methoxycinnamate (EMC), increased with the decrease in pH. Our research group [11] also obtained and reported similar findings for EMC and also another UV filter, 4-*tert*-butyl-4'-methoxydibenzoylmethane (BDM). According to the pH-dependence of the hypochlorous acid/hypochloride anion (HOCl/ClO^-) equilibrium ($\text{pK}_a = 7.54$, at 25°C) [19], the hypochloride anion clearly predominates at higher levels of pH, namely at $\text{pH} > 7.5$, whereas hypochlorous acid, which predominates between $4 < \text{pH} < 7.5$ [17, 19], is usually defined as the most efficient chlorine disinfecting agent [19]. The results of the present study, seem to point to a significant and sequential degradation of the non-volatile chlorinated by-products into smaller and more volatile molecules, undetected in the LC-DAD-MS analysis. This may explain why the highest concentration of non-volatile DBPs, is found at higher pH and lower concentrations of active chlorine. At higher pH values, hypochloride anion, considered a low efficiency disinfecting agent, clearly predominates, and together with lower levels of active chlorine in solution, seems to lead to a decrease in the degradation rate of the non-volatile DBPs. Additionally, detection of higher levels of chlorinated DBPs at higher pH values, also points to reactions of the ionized form of DHHB with hypochloride anion, suggesting that the ionized form of the parental compound may in fact predominate in solution. The effective degradation of DHHB, that is, the detected or accounted total loss of DHHB concentration, presented in Figure S13, points to those same conclusions. Degradation of DHHB is indeed found

to be much more significant at higher levels of active chlorine in solution and lower pH values, conditions in which the considered more efficient hypochlorous acid predominates. The chlorination of organic compounds is precisely what leads to their degradation, so following the variation in chlorinated DBP concentrations and the establishment of [Figure S12](#) for analysis, was merely important to identify what seems to point towards the instability of these non-volatile by-products (and the inherent increase in their degradation rates) at higher levels of active chlorine and higher pH values.

In Batch B, another design variable (DOM) was included, in order to ascertain the effect of conventional organic matter, usually found dissolved in natural and artificial water environments, on the chlorination and degradation reactions. In a similar way to the results from Batch A, data shows that pH is the most significant variable to influence both the chlorination and effective degradation of DHHB. As far as DOM is concerned, it is known that it actively competes with any synthetic organic compounds in solution, for the available free active chlorine. Subsequently, it was expected that chlorination and degradation rates would decrease with the increase in DOM levels in solution. However, DOM proved to have no negative effect on the degradation of DHHB, quite possibly due to the highly reactive nature of DHHB towards free available chlorine. [Figures S14-S15](#), present in the supplementary material, display the response surfaces for the chlorination and effective degradation of DHHB, respectively, by the combination of the two most significant variables of Batch B, pH and NaOCl concentration. Analysis of [Figures S14-S15](#) show the same trend as in Batch A, defining that degradation of DHHB is, indeed, highlighted at higher levels of chlorine in solution and lower levels of pH.

3.2. Photo-degradation studies

As referred to in the Introduction of this article, merely one study [\[8\]](#) focused simultaneously on the disinfection-induced and photo-induced degradation of UV filters. In light of that fact, it was the goal of the present work to address those two degradation contexts. That being said, it is important to underline that photo-induced degradation of certain UV filters has been the specific focus of several studies [\[21-24\]](#).

The UV filter DHHB, also known commercially by the trivial name Uvinul A Plus, is officially described as being “outstandingly photostable” [\[20\]](#). The purpose of this section of the work, was to test that designation and ascertain the stability of the

compound when exposed to artificial solar radiation, and potentially determine some photo-degradation by-products, if detected.

Results have shown that DHHB concentration decreased less than 15% from the initial levels of the compound, and after the course of 6 hours of exposure to artificial solar radiation. This degradation rate should be considered fairly negligible in the scope of the overall photo-stability of the compound. At every step of the study, an analysis by HPLC-UV-DAD was always carried out, in order to detect possible by-products. No peaks, besides the one from the filter, were ever detected by liquid chromatography (HPLC-UV-DAD and LC-DAD-MS). Therefore, it must be concluded that this very small amount of degradation rate detected throughout the experiments, is not significant to yield any measurable amounts of possible by-products. These results are, for instance, identical to those found by Rodil [21], for filters such as 4-methylbenzylidene camphor (MBC), benzophenone-3 (BP3) or octocrylene (OC), which were also studied in similar parameters and displayed considerable photo-stability throughout the considered period of artificial irradiation.

Parallel to the chromatographic analysis, a spectroscopic study was also carried out, in order to measure the potential alterations in the UV absorption capacity of DHHB along with the irradiation time. Figure 7 presents the UV absorption spectra of the artificially-irradiated solution. Analysis of the spectra indicates that there is a very slight decrease in the absorption capacity of the irradiated solution. More thoroughly, though, using Maier's concept of spectral photo-inactivation [22], that is, the difference between the absorbance capacity before and after irradiation, will denote an absorption capacity decay of merely 14.73%, after the corresponding period of 6 hours of irradiation. These results demonstrate that DHHB does display remarkable photo-stable characteristics, when exposed towards artificial solar radiation. Despite the fact that the DHHB aqueous solutions were able to remain photo-stable throughout the irradiation reaction, there was one photo-exposure effect often observed in photo-degradation studies [23, 24]: a main absorption wavelength shift, in this case from the conventional 361 nm prior to irradiation, towards 375-377 nm after the irradiative process. In the global scope of the UV absorption features of DHHB, the wavelength shift and absorption capacity decrease are considered minor and therefore very much negligible regarding the UV-protective character of the filter.

4. Conclusions

The UV filter DHHB proved to be extremely reactive towards active chlorine and in drinking water disinfection conditions. The kinetic assessment of the degradation reaction, allowed to prove that fact, with the determination of a half-life period quite low of 116 ± 3 seconds and an observed first-order rate constant of $0.0060 \pm 0.0002 \text{ s}^{-1}$. The degradation reaction yielded mono (Cl-DHHB) and dichlorinated (Cl_2 -DHHB) DBPs of the parental structure, successfully determined by MS analysis.

In the study of the influence of external factors on degradation, pH and free chlorine levels proved to be the significantly most influential variables, either in the presence or absence of DOM in solution. Degradation rates of DHHB were found, as expected, at higher levels of active chlorine in solution and at lower pH values. Additionally, it was found that the chlorinated by-products seemed to prevail in the opposite conditions, that is, lower levels of active chlorine and higher pH. This fact points to the likely possibility of these non-volatile chlorinated DBPs being unstable when present in higher concentrations of chlorine as well as lower pH, leading to their sequential degradation and formation of volatile compounds undetected in the chromatographic analysis.

As for the photo-degradation studies, DHHB proved to display the features that are actually used to commercially describe and promote the compound, that is, outstandingly photo-stable. Degradation rates observed were considered to be negligible, around 15% in 6 hours of exposure to artificial solar radiation, and no by-products were ever detected as well. Spectroscopic data suggest that there is a slight and not very relevant decrease in the overall absorption capacity of the filter, as well as a minor absorption wavelength shift from the original 360-361 nm towards 375-377 nm. These findings are, however, not considered to remotely hinder the filter's UV-protective abilities, and therefore it is concluded that DHHB is indeed remarkably photo-stable.

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Figures

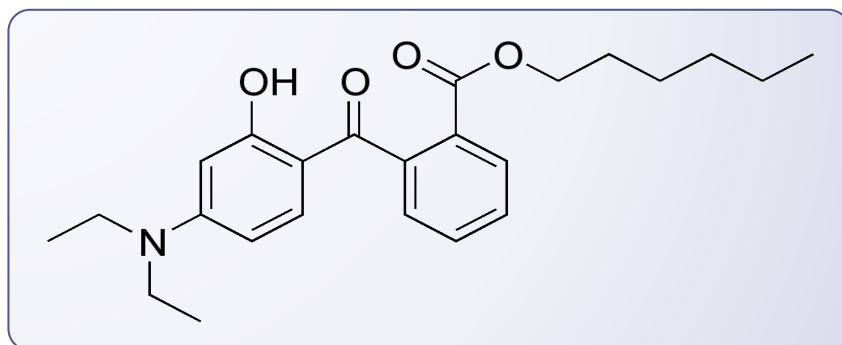


Figure 1. Structure of hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB).

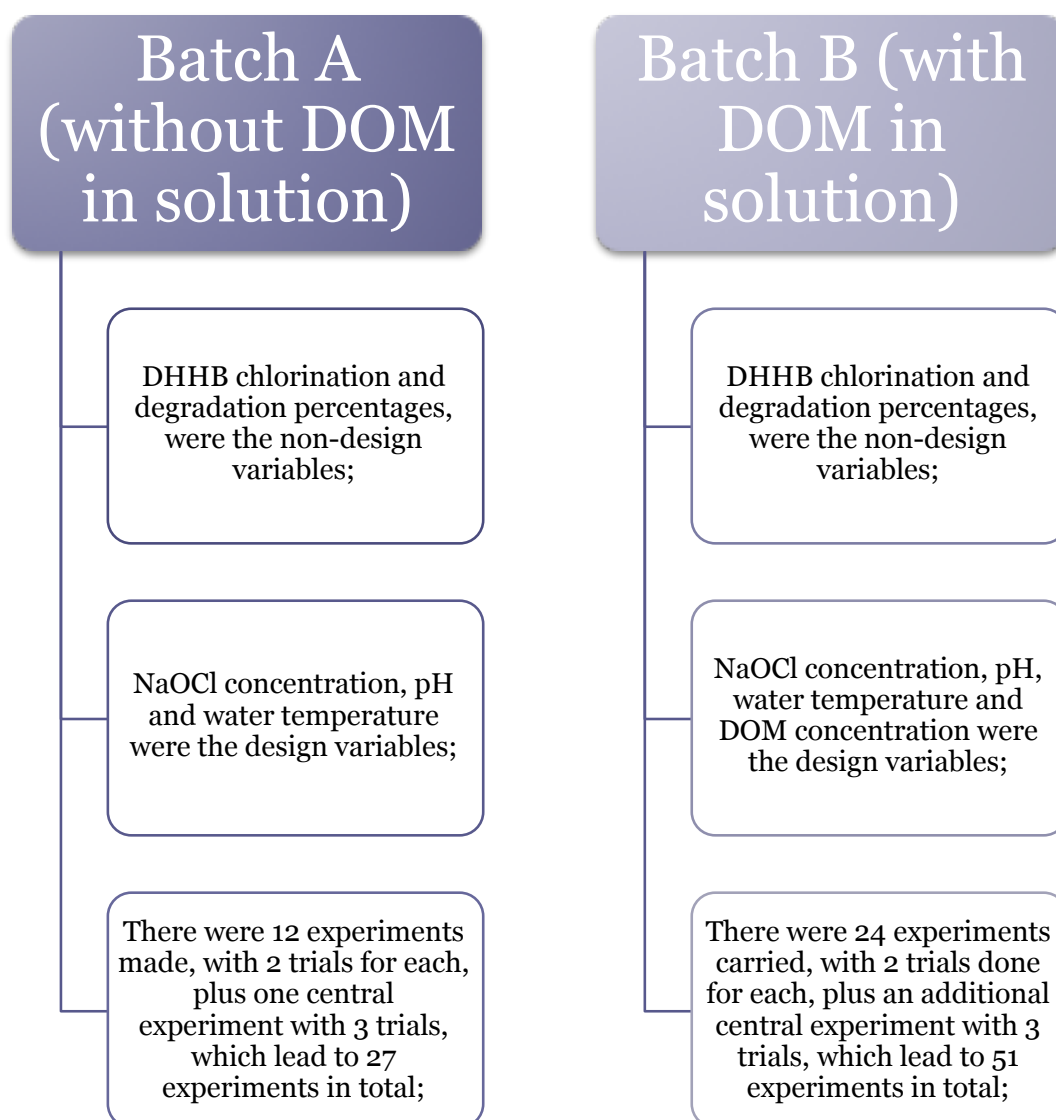


Figure 2. Details on the two Box-Behnken designs created and studied, Batch A and Batch B.

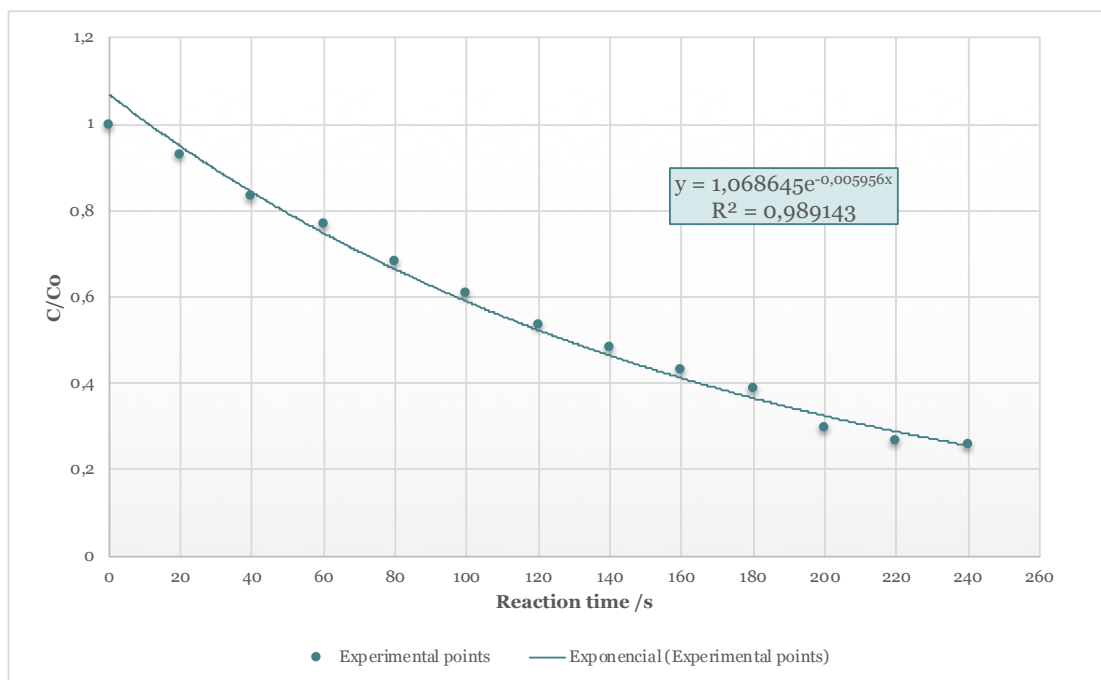


Figure 3. Exponential plot of DHHB degradation with NaOCl, as a function of reaction time.

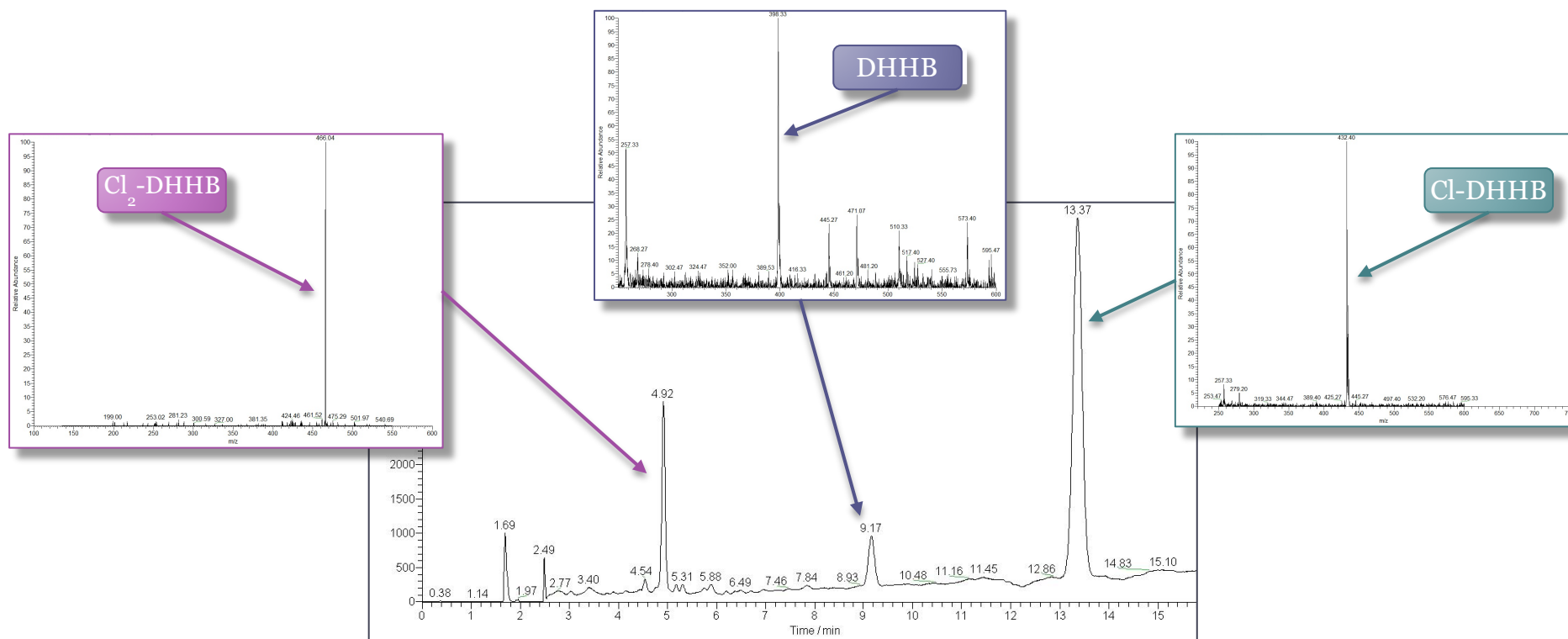


Figure 4. Identification of DBPs from the reaction of DHHB with NaOCl at neutral pH and in the dark.

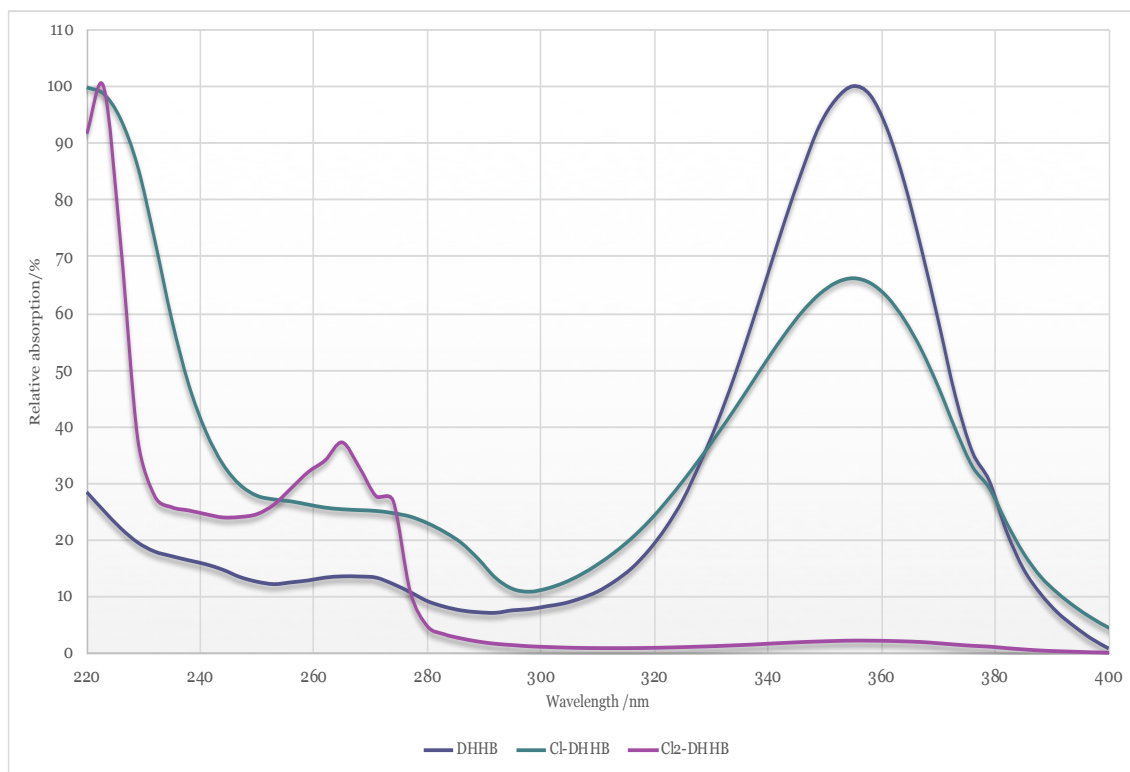


Figure 5. UV absorption spectra of DHHB and its mono and dichlorinated DBPs.

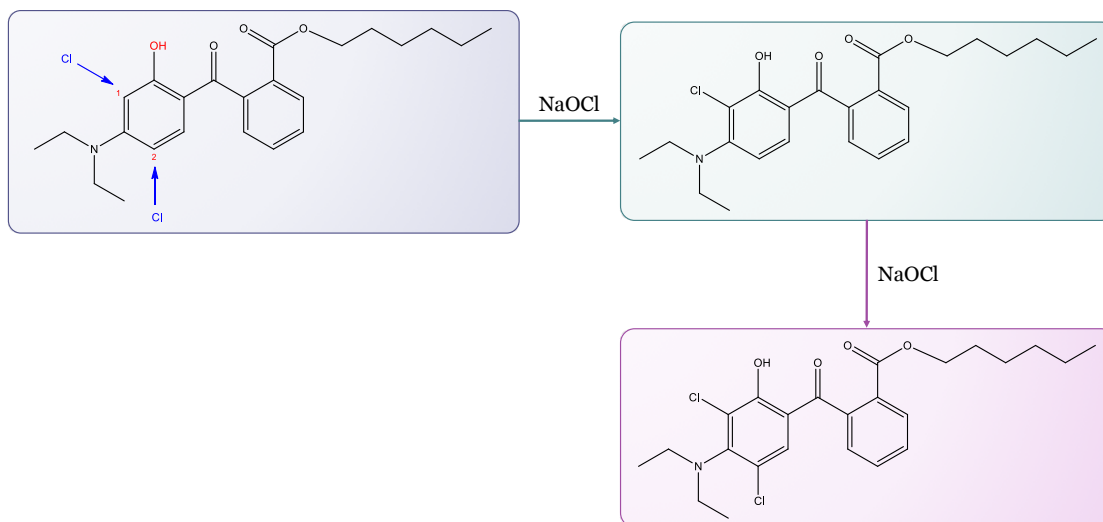


Figure 6. Representation of the sites prone to the electrophilic substitution of chlorine in the DHHB compound (*ortho* and *para* position towards the hydroxyl group in red), as well as the structure of the determined DBPs.

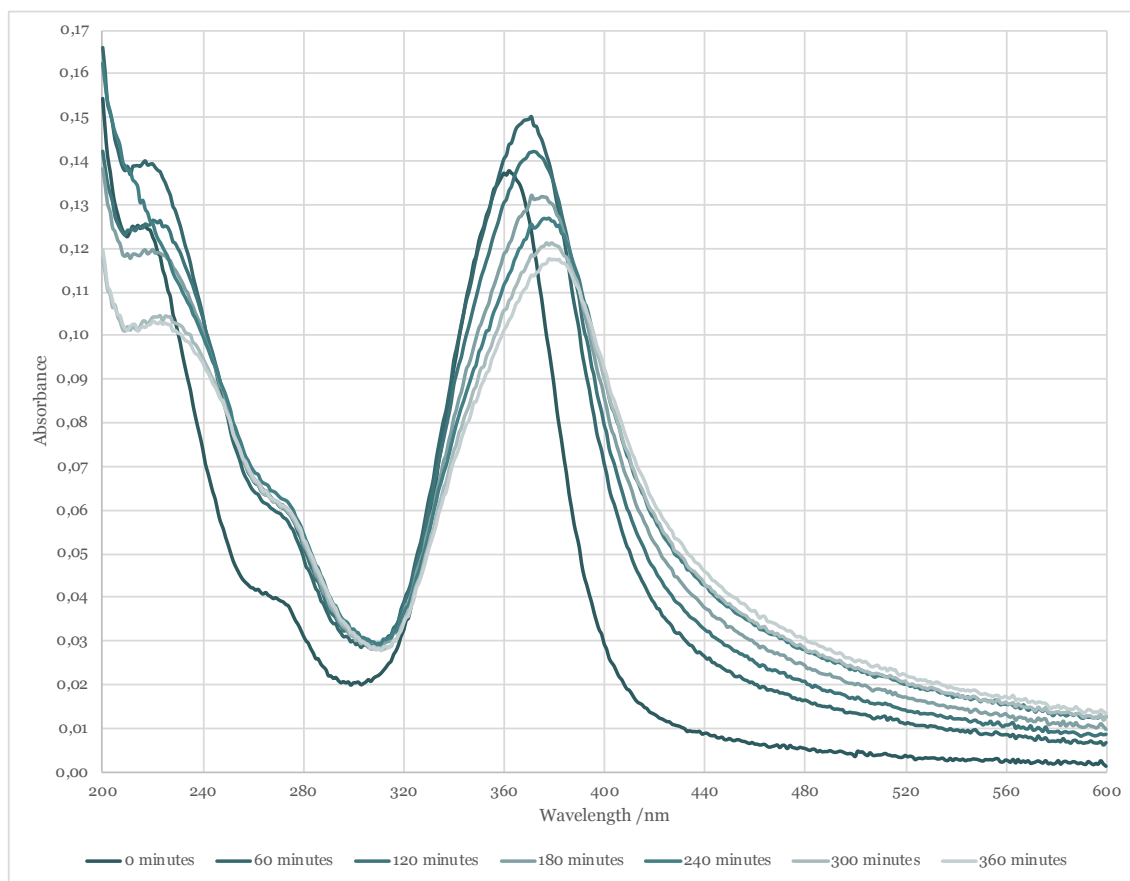


Figure 7. UV absorbance spectra of the artificially-irradiated DHHB solution.

Tables

Table 1. Physical-chemical properties of DHHB.

IUPAC name	Acronym	Regulation	UV range	$\lambda_{\text{m\acute{a}x}}$ /nm	ϵ /L mol ⁻¹ cm ⁻¹	Molecular mass	Log K _{ow} †	Solubility g/L*
Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate	DHHB	The filter is currently regulated in the EU and Japan	UVA	355-356	35.900	397.5073	6.93	9.5x10 ⁻⁴

* Determined in water and at 25 °C, according to reference [5].

† Octanol water partition coefficient (K_{ow}); represents the ratio of the concentration of a substance in octanol and the concentration of the same substance in equilibrium in water, at a certain temperature.

Table 2. LC-DAD-MS results from the analysis of the degradation reactions of DHHB with active chlorine.

Retention time /min	Identification	Molecular ion /m/z	Fragmentation ions /m/z (relative intensity /%)	Proposed molecular formula	Abbreviation
4.92	[DHHB+Cl ₂ +H] ⁺	466.04	149.20 (100); 232.82 (52); 364.01 (48);	C ₂₄ H ₃₀ NO ₄ Cl ₂ ⁺	Cl ₂ -DHHB
9.17	[DHHB+H] ⁺	398.33	149.20 (100); 232.93 (42); 296.27 (24); 189.87 (10);	C ₂₄ H ₃₂ NO ₄ ⁺	DHHB
13.37	[DHHB+Cl+H] ⁺	432.40	330.20 (100); 302.33 (22); 348.07 (16); 284.07 (14); 274.40 (11);	C ₂₄ H ₃₁ NO ₄ Cl ⁺	Cl-DHHB

Supplementary material

Fate and behaviour of the UV filter hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate (DHHB) in aqueous solution

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1. Identification of DBPs

Figures S1-S4 present the proposed molecular structures for the identified DBPs of DHHB. Figures S5-S11 present the mass spectra and corresponding first and second fragmentations, if necessary, for DHHB and its mono and dichlorinated DBPs. Mass spectra details for the peaks from Figure 4 of the main article are as follows (relative abundance values are mentioned in brackets):

- Peak at 9.17 minutes (identified as the parental compound, DHHB): mass spectrum yielded the m/z value of 398.33 (100%), consistent with the molecular mass of the filter;
 - Fragmentation of this peak originated the m/z values of 149.20 (100%), 232.93 (43%), 296.27 (24%) and 189.87 (10%) as the most significant ions;
- Peak at 13.37 minutes (identified as the monochlorinated form of DHHB, Cl-DHHB): the mass spectrum of this peak showed a m/z value of 432.40 (100%), consistent with the molecular mass of monochlorinated DHHB;
 - First fragmentation of this peak yielded the m/z values 330.20 (100%), 302.33 (22%), 348.27 (16%), 284.07 (15%), 274.40 (12%) and 403.07 (6%);
 - Second fragmentation originated the m/z values 302.13 (100%), 266.27 (10%), 223.20 (6%) and 266.33 (9%);
- Peak at 4.92 minutes (identified as the dichlorinated form of DHHB, Cl₂-DHHB): the corresponding mass spectrum of the peak yielded the m/z value 466.04 (100%), which is consistent with the molecular mass of dichlorinated DHHB:
 - Fragmentation of the peak led to m/z values of 149.20 (100%), 232.82 (53%), 364.01 (48%), 318.24 (6%), 189.51 (6%), 132.63 (5%), 350.18 (5%) and 375.12 (5%);

The results obtained in the peak's mass spectra, as well as the fragmentations carried out, are compatible with the results reported by Grbović [12] for the same compound and respective DBPs.

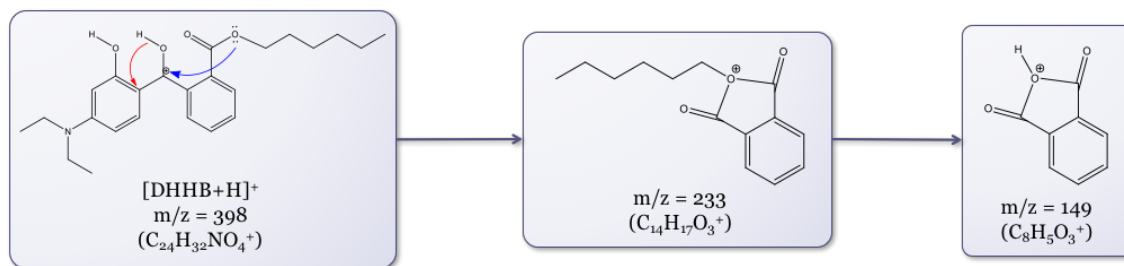


Figure S1. Fragmentation of [DHHB + H]⁺ into two of the most predominant molecular ions, m/z = 233 and 149 (adapted from [12]).

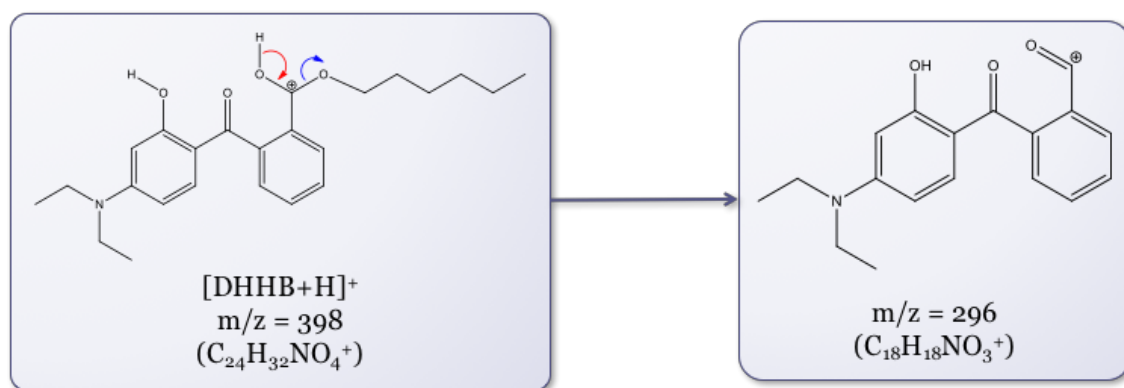


Figure S2. Fragmentation of [DHHB + H]⁺ into the remaining most predominant molecular ion, m/z = 296 (adapted from [12]).

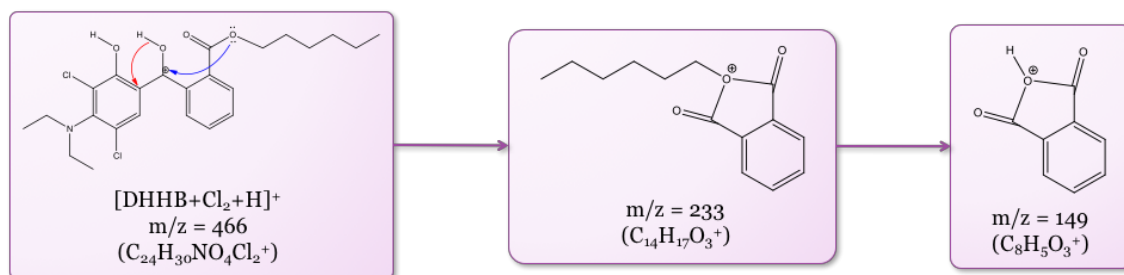


Figure S3. Fragmentation of [DHHB + Cl₂ + H]⁺ into its most prominent molecular ions, m/z = 233 and 149 (adapted from [12]).

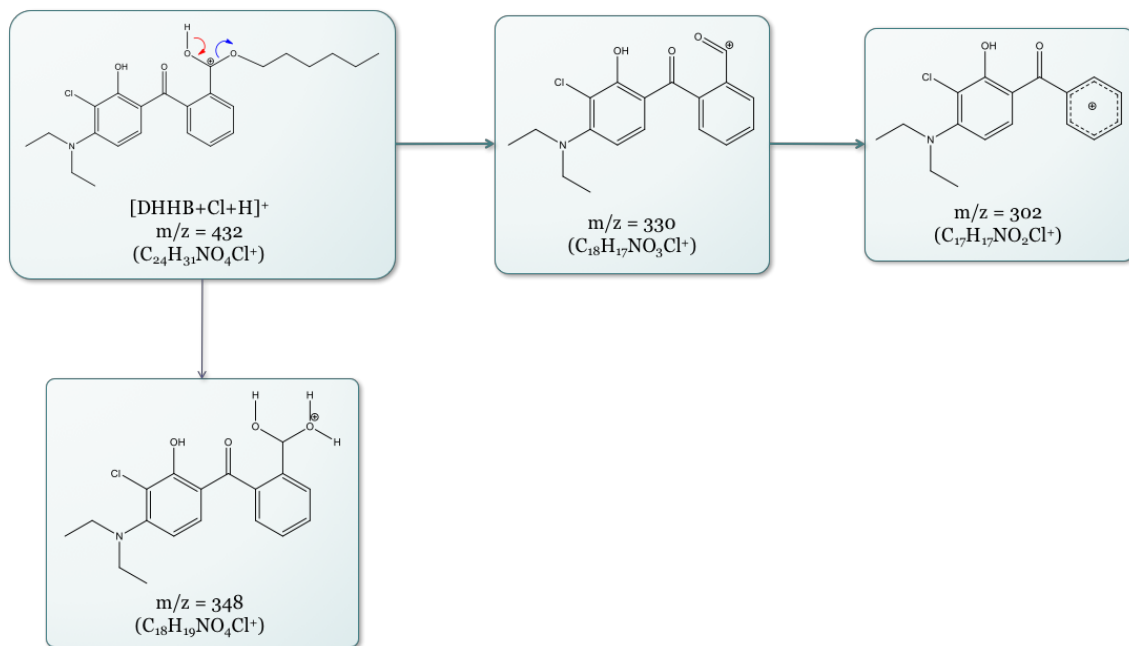


Figure S4. Fragmentation of [DHHB + Cl + H]⁺ into its most significant molecular ions, m/z = 330, 302 and 348 (adapted from [12]).

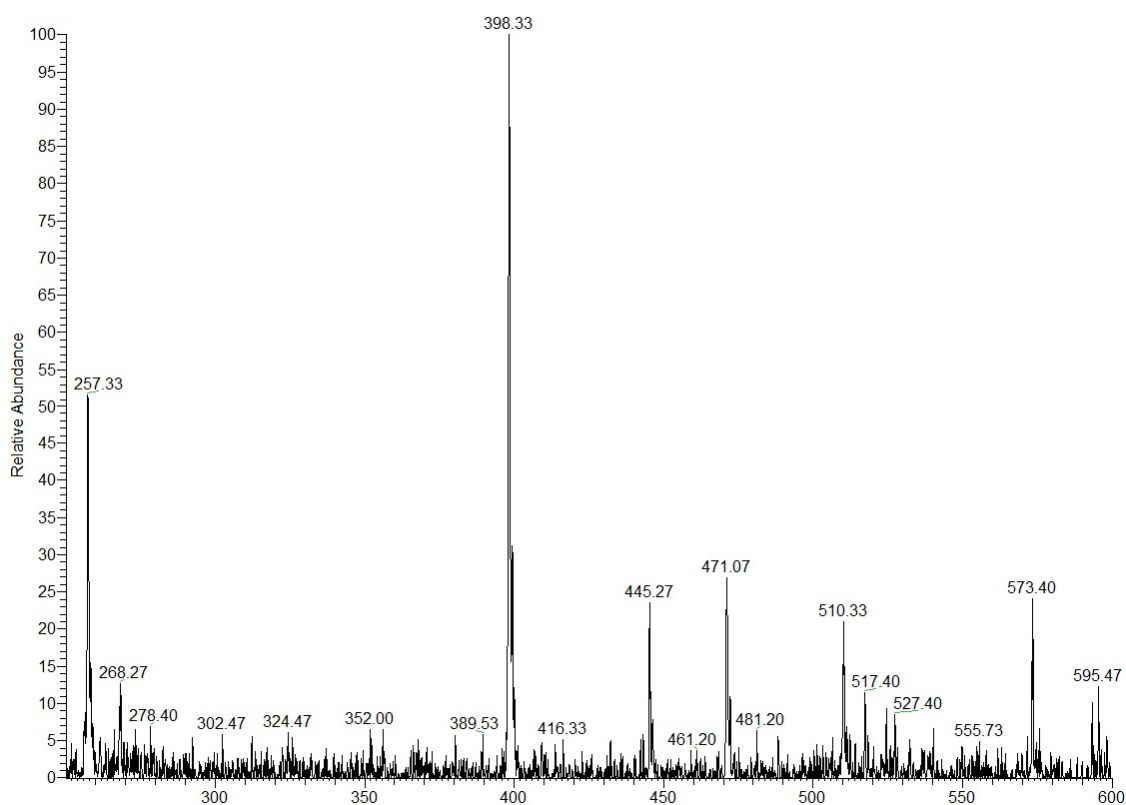


Figure S5. Mass spectrum of the parental compound, DHHB.

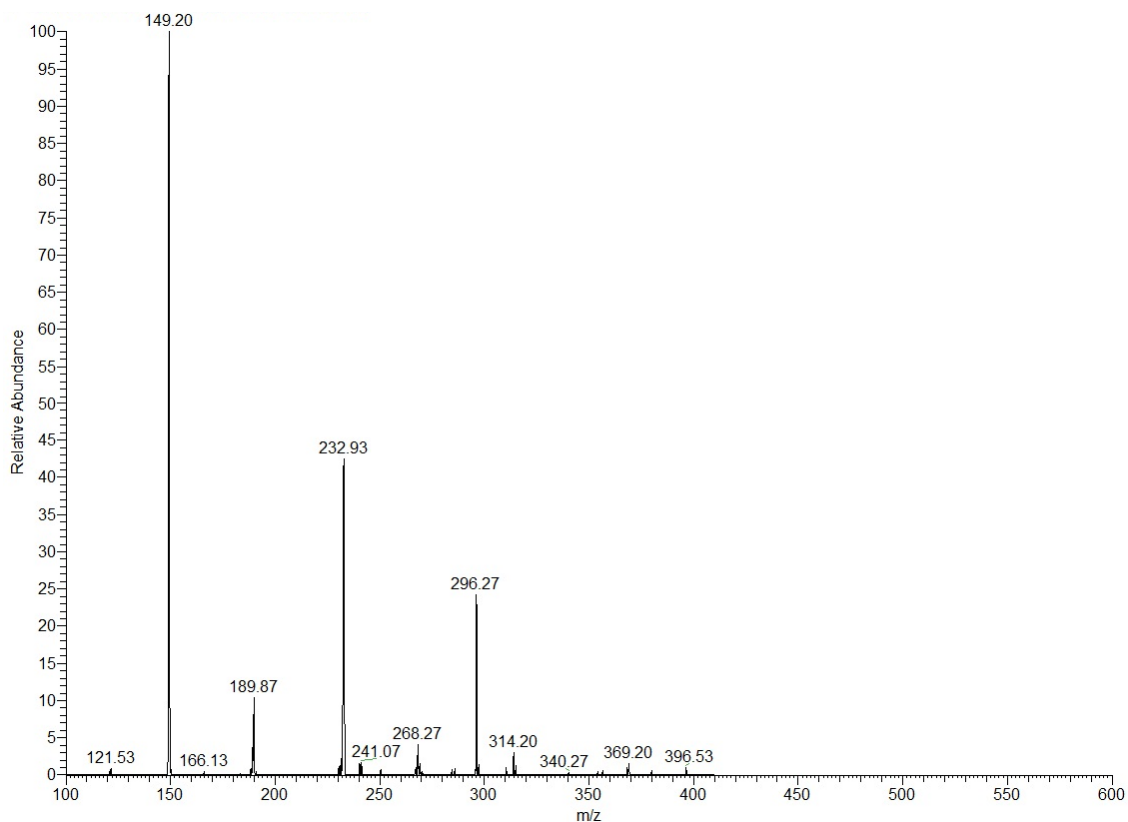


Figure S6. Fragmentation of DHHB respective fragments ions.

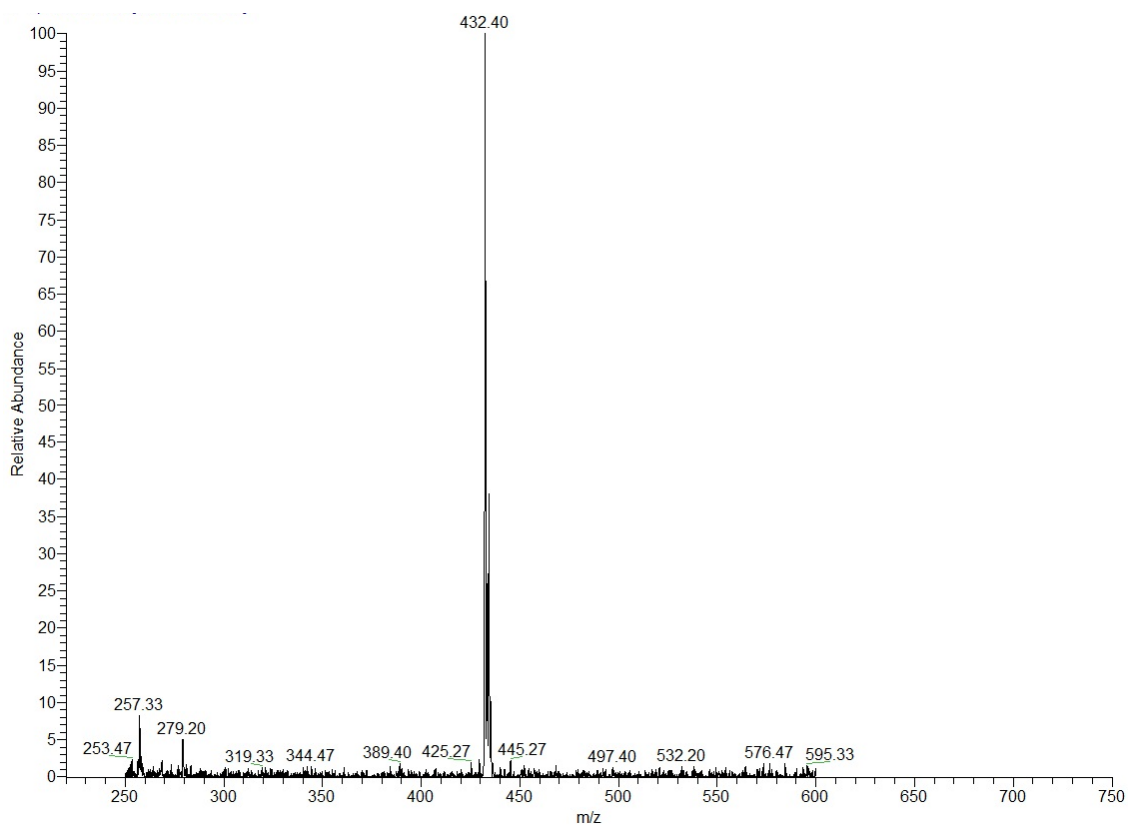


Figure S7. Mass spectrum of the monochlorinated form of DHHB (Cl-DHHB).

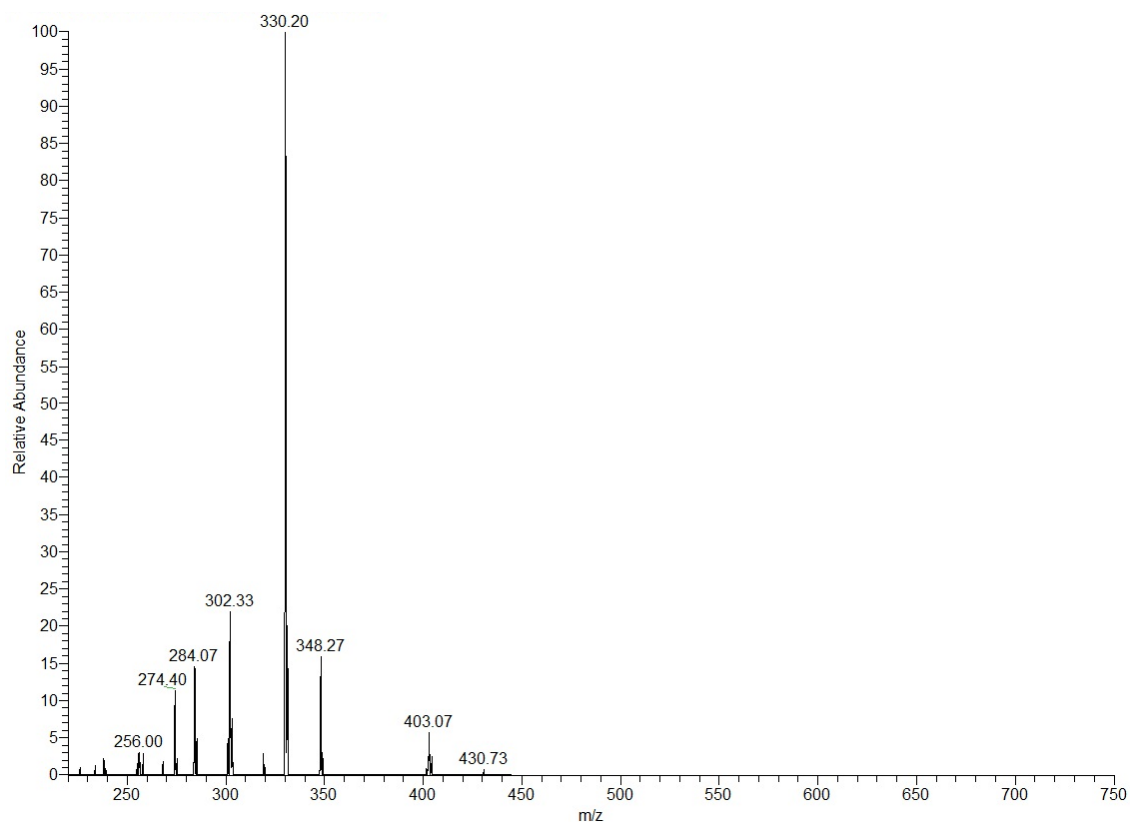


Figure S8. First fragmentation of Cl-DHNB and respective fragment ions.

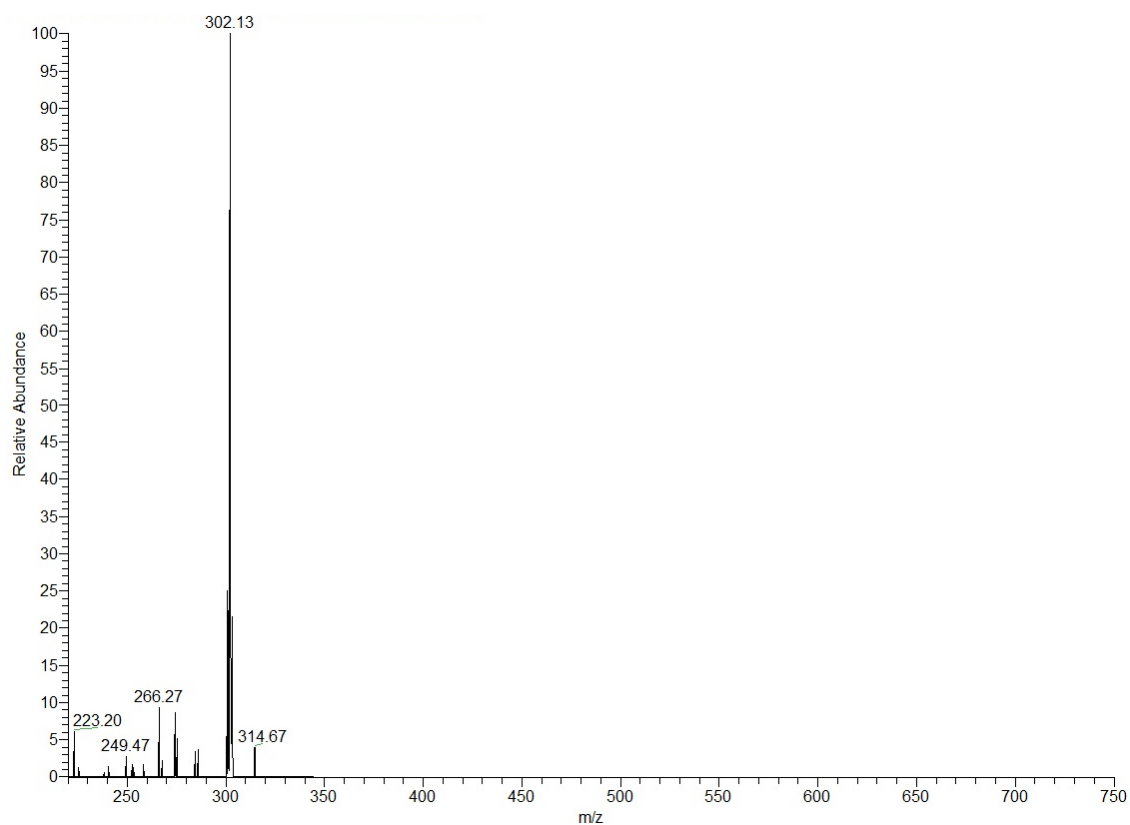


Figure S9. Second fragmentation of Cl-DHNB and respective fragment ions.

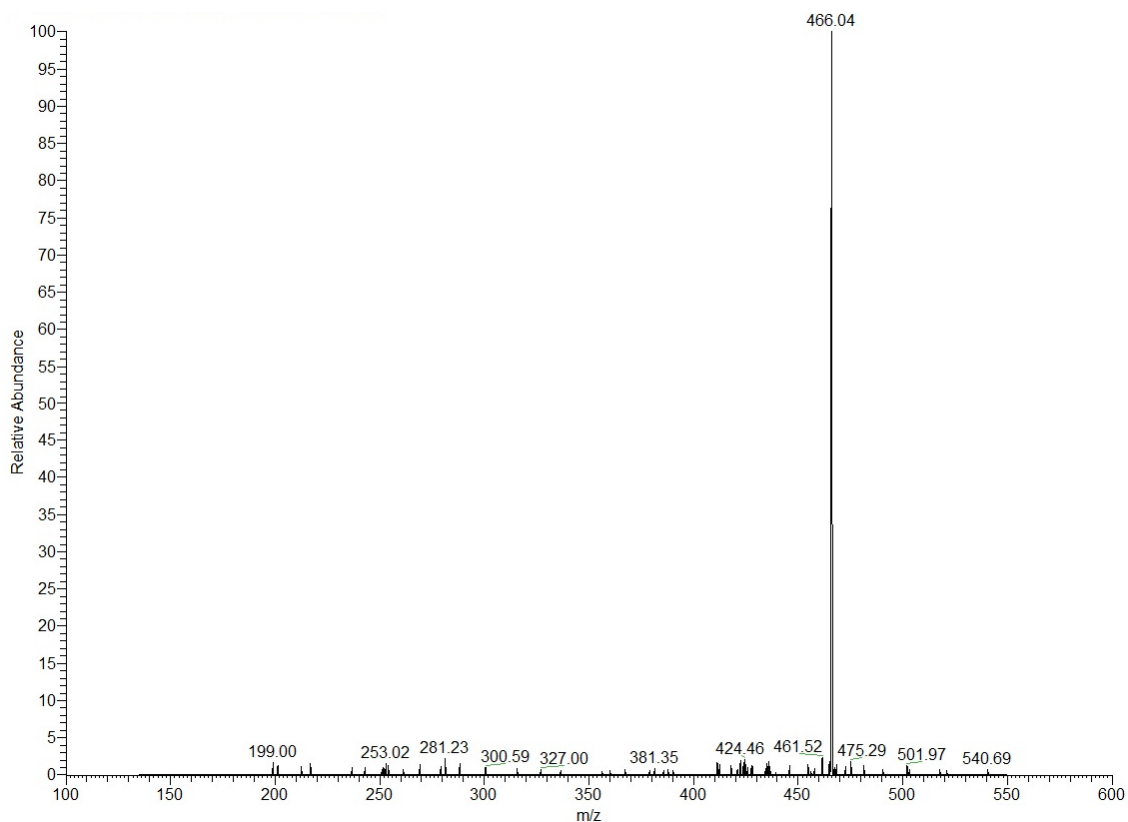


Figure S10. Mass spectrum of the dichlorinated form of DHHB ($\text{Cl}_2\text{-DHHB}$).

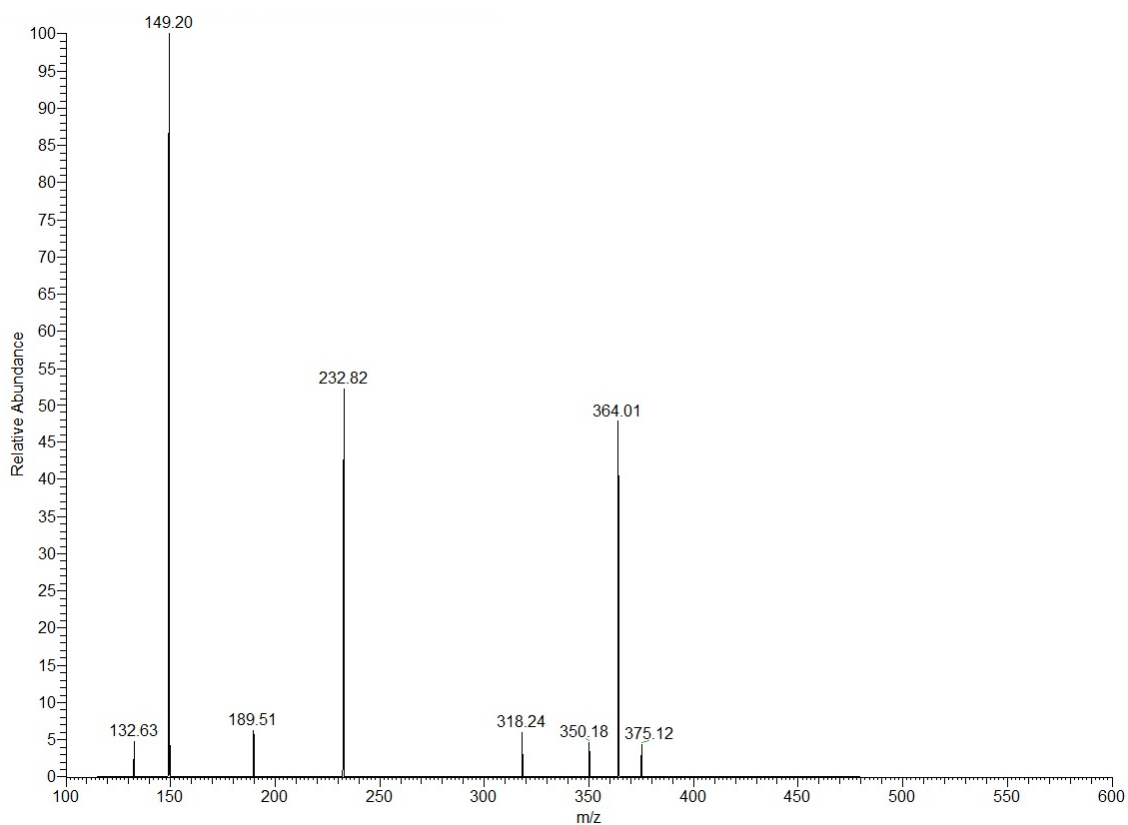


Figure S11. Fragmentation of $\text{Cl}_2\text{-DHHB}$ and respective fragment ions.

2. Influence of external variables on degradation

Table S1. Experimental conditions for the Batch A of experiments.

Experiment	[DHHB] /mg/L	[NaOCl] /mg/L	pH	T /°C
1	0.8000	1.000	6	20
1b	0.8000	1.000	6	20
2	0.8000	3.000	6	20
2b	0.8000	3.000	6	20
3	0.8000	1.000	8	20
3b	0.8000	1.000	8	20
4	0.8000	3.000	8	20
4b	0.8000	3.000	8	20
5	0.8000	1.000	7	15
5b	0.8000	1.000	7	15
6	0.8000	3.000	7	15
6b	0.8000	3.000	7	15
7	0.8000	1.000	7	25
7b	0.8000	1.000	7	25
8	0.8000	3.000	7	25
8b	0.8000	3.000	7	25
9	0.8000	2.000	6	15
9b	0.8000	2.000	6	15
10	0.8000	2.000	8	15
10b	0.8000	2.000	8	15
11	0.8000	2.000	6	25
11b	0.8000	2.000	6	25
12	0.8000	2.000	8	25
12b	0.8000	2.000	8	25
Central a	0.8000	2.000	7	20
Central b	0.8000	2.000	7	20
Central c	0.8000	2.000	7	20

Table S2. Experimental conditions for the Batch B of experiments.

Experiment	[DHHB] /mg/L	[NaOCl] /mg/L	[DOM] /mg/L	pH	T /°C
1	0.8000	1.000	2.000	6	20
1b	0.8000	1.000	2.000	6	20
2	0.8000	3.000	2.000	6	20
2b	0.8000	3.000	2.000	6	20
3	0.8000	1.000	2.000	8	20
3b	0.8000	1.000	2.000	8	20
4	0.8000	3.000	2.000	8	20

4b	0.8000	3.000	2.000	8	20
5	0.8000	1.000	2.000	7	15
5b	0.8000	1.000	2.000	7	15
6	0.8000	3.000	2.000	7	15
6b	0.8000	3.000	2.000	7	15
7	0.8000	1.000	2.000	7	25
7b	0.8000	1.000	2.000	7	25
8	0.8000	3.000	2.000	7	25
8b	0.8000	3.000	2.000	7	25
9	0.8000	1.000	1.000	7	20
9b	0.8000	1.000	1.000	7	20
10	0.8000	3.000	1.000	7	20
10b	0.8000	3.000	1.000	7	20
11	0.8000	1.000	3.000	7	20
11b	0.8000	1.000	3.000	7	20
12	0.8000	3.000	3.000	7	20
12b	0.8000	3.000	3.000	7	20
13	0.8000	2.000	2.000	6	15
13b	0.8000	2.000	2.000	6	15
14	0.8000	2.000	2.000	8	15
14b	0.8000	2.000	2.000	8	15
15	0.8000	2.000	2.000	6	25
15b	0.8000	2.000	2.000	6	25
16	0.8000	2.000	2.000	8	25
16b	0.8000	2.000	2.000	8	25
17	0.8000	2.000	1.000	6	20
17b	0.8000	2.000	1.000	6	20
18	0.8000	2.000	1.000	8	20
18b	0.8000	2.000	1.000	8	20
19	0.8000	2.000	3.000	6	20
19b	0.8000	2.000	3.000	6	20
20	0.8000	2.000	3.000	8	20
20b	0.8000	2.000	3.000	8	20
21	0.8000	2.000	1.000	7	15
21b	0.8000	2.000	1.000	7	15
22	0.8000	2.000	1.000	7	25
22b	0.8000	2.000	1.000	7	25
23	0.8000	2.000	3.000	7	15
23b	0.8000	2.000	3.000	7	15
24	0.8000	2.000	3.000	7	25
24b	0.8000	2.000	3.000	7	25
Central a	0.8000	2.000	2.000	7	20
Central b	0.8000	2.000	2.000	7	20
Central c	0.8000	2.000	2.000	7	20

Tables S3 and S4 present the ANOVA of chlorination and degradation models respectively, for the Batch A of experiments with NaOCl concentration, pH, and temperature, along with their corresponding quadratic terms and interactions. First and foremost, it is imperative to ascertain the validity of the models. Thus, for the chlorination model of the first design:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the theoretical *F*-value (obtainable from the distribution tables of statistical literature) is 1.833;
- As presented in Table S3, the experimental *F*-value of the model for the chlorination of DHHB is 49.780, and therefore, significantly higher than the theoretical value above;
- Additionally, the *P*-value for the model is 0, and therefore lower than the significance level ($\alpha = 0.05$);

This data allows the conclusion that the model is statistically valid or presents statistical significance. As for the variables under study, the NaOCl (β -coefficient = -14,119) and pH (β -coefficient = 19.401) proved to be the most significant for the chlorination of DHHB. In parallel, these are also the factors with the highest *F*-values (127.325 for NaOCl concentration, and 240.410 for the pH). As for the *P*-values, all three factors fall below the significance level of 5%, but NaOCl concentration and pH also display the lowest (0 for both variables, and 0.0003 for the temperature). Regarding the second order or interaction terms, the quadratic term pH x pH displays the highest significance with an *F*-value of 35.102 and a *P*-value of 0.

In the case of the effective degradation within Batch A of experiments, the model's statistical features are as follows:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the theoretical *F*-value (obtained from the conventional distribution tables of statistical literature) is 1.833;
- As presented in Table S4, the experimental *F*-value of the model for the effective degradation of DHHB is 43.734, and therefore, significantly higher than the theoretical value;
- The *P*-value of the model is 0, and therefore lower than the significance level ($\alpha = 0.05$);

In light of the data presented, the model for the effective degradation of DHHB in the first design of experiments is considered valid or statistically significant. Regarding the most

significant factors to contribute to the degradation of DHHB, the NaOCl concentration (β -coefficient = 14.887) and pH (β -coefficient = -18.896) proved again to be most prominent. These are also the variables with the highest F -values (123.674 for the NaOCl concentration, and 199.247 for the pH) and lowest P -values (0 for the NaOCl concentration and pH and 0.0003 for the temperature). The second order or interaction term statistically significant the most, is the factor pH x pH with an F -value of 32.999 and a P -value of 0.

As for the Batch B of experiments, [Tables S5 and S6](#) present the ANOVA for the chlorination an effective degradation models respectively, with NaOCl concentration, pH, temperature, DOM concentration and their quadratic terms and interactions, as the design variables. The characteristics of the chlorination model of Batch B of experiments are the following:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the theoretical F -value (obtained from the conventional distribution tables of statistical literature) is 1.761;
- As presented in [Table S5](#), the experimental F -value of the model for the chlorination of DHHB is 33.758, and therefore, significantly higher than the theoretical value;
- The P -value of the models is 0, and therefore, lower than the significance level ($\alpha = 0.05$);

Consequently, it must be concluded that the chlorination model of the second design of experiments is valid or statistically significant. The most significant design variables proved to be again the NaOCl concentration (β -coefficient = -15.287) and pH (β -coefficient = 17.659). These factors also displayed the highest F -values (156.969 for the NaOCl concentration and 209.463 for the pH) and lowest P -values (0 for both factors). DOM, which is known to actively compete with synthetic organic compounds for the available free chlorine in solution, showed to have very little to no effect whatsoever (β -coefficient = 1.642), and displayed an F -value (1.810) only slightly higher than the theoretical one (1.761), while the P -value (0.1869) was actually higher than the significance level ($\alpha = 0.05$). The interaction term NaOCl x T (β -coefficient = -4.357; F -value = 18.445; and P -value = 0.0001) and quadratic term pH x pH (β -coefficient = 5.004; F -value = 22.466; and P -value = 0), both demonstrated to have some influence on the chlorination of DHHB.

In the model for the effective degradation of Batch B of experiments, the statistical data are as follows:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the theoretical F -value (obtained from the conventional distribution tables of statistical literature) is 1.761;
- As presented in Table S6, the experimental F -value of the model for the degradation of DHHB is 35.146, and therefore, significantly higher than the theoretical value;
- The P -value of this model is 0, and therefore, lower than the significance level ($\alpha = 0.05$);

Thus, it is possible to conclude that the effective degradation model of the second design of experiments is valid and presents statistical significance. Like in the precedent cases, the variables which demonstrated higher statistical prominence in the degradation of DHHB, proved to be the NaOCl concentration (β -coefficient = 15.911) and the pH (β -coefficient = -16.993). The lowest F -values were also presented by these factors (177.319 for the NaOCl concentration and 202.258 for the pH). The lowest P -values also followed the same trend, being displayed by NaOCl concentration and pH (0 for both variables). As for the interaction or quadratic terms' influence, the interactive term NaOClxT (β -coefficient = 4.084; F -value = 16.904; and P -value = 0.0002) and the quadratic term pHxpH (β -coefficient = -5.279; F -value = 26.064; and P -value = 0) proved to be statistically significant in the degradation of DHHB.

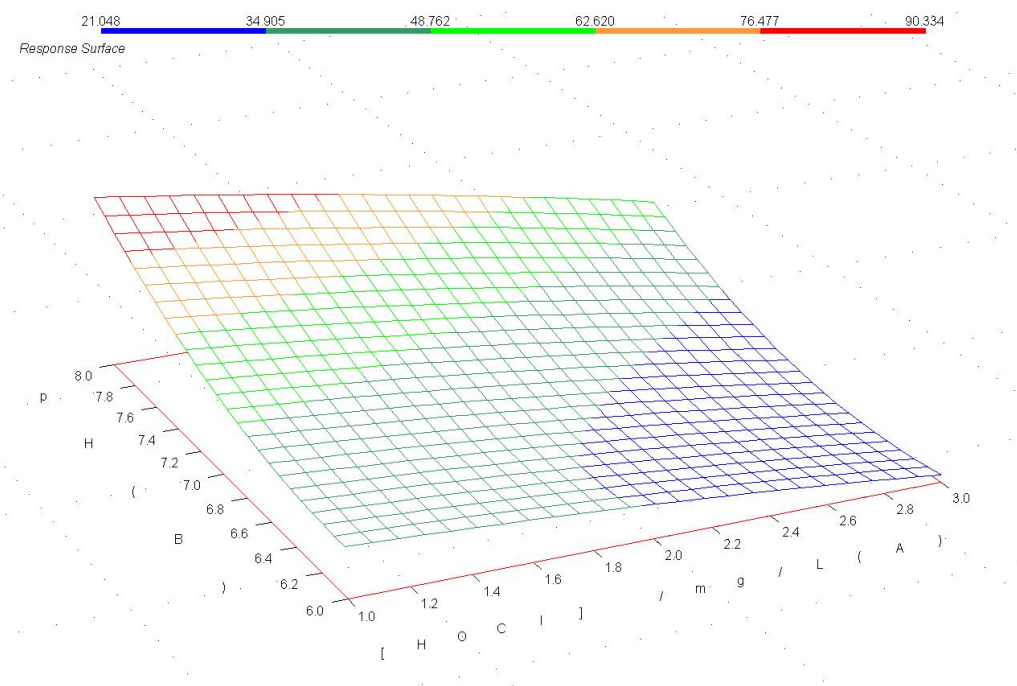


Figure S12. Response surface for the chlorination percentage variation as a function of pH and NaOCl concentration, in Batch A.

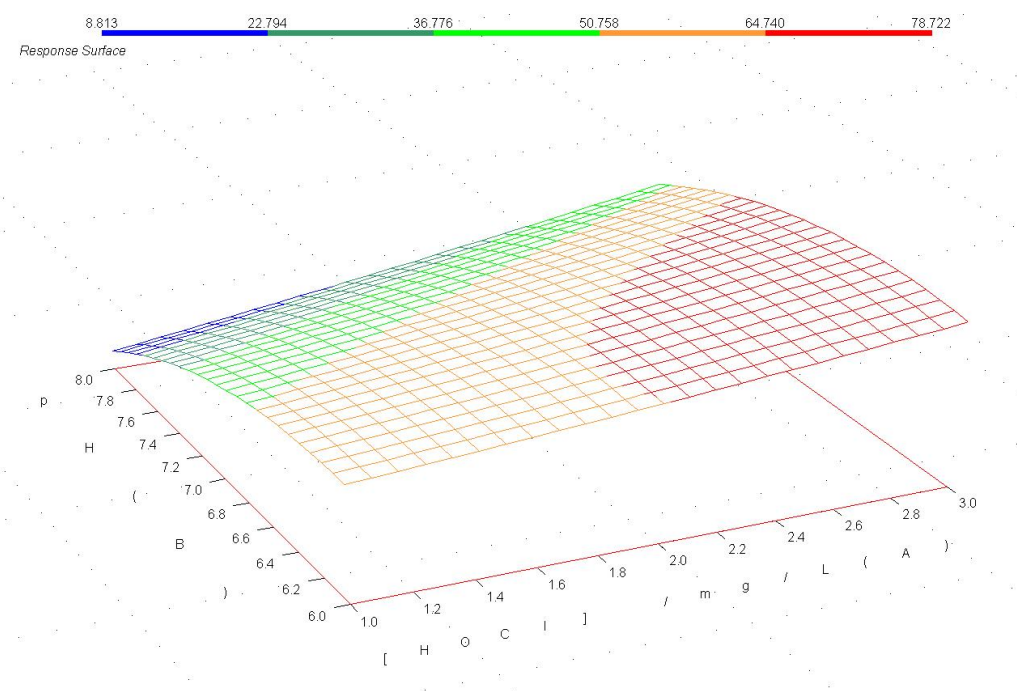


Figure S13. Response surface for the effective degradation percentage variation as a function of pH and NaOCl concentration, in Batch A.

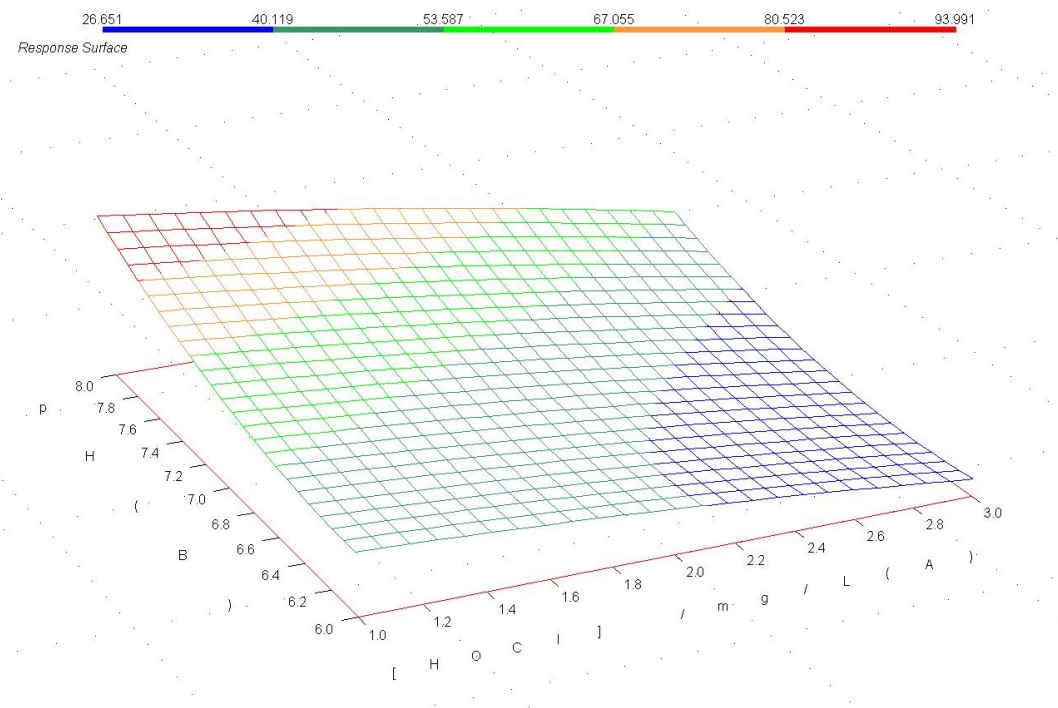


Figure S14. Response surface for the chlorination percentage variation as a function of pH and NaOCl concentration, in Batch B.

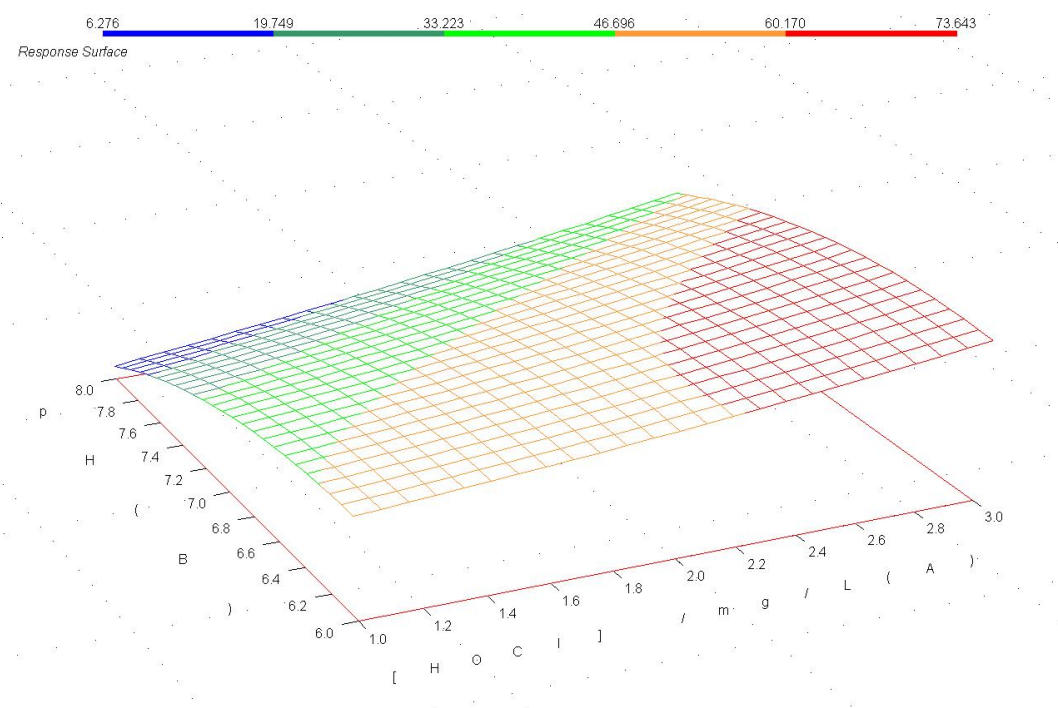


Figure S15. Response surface for the chlorination percentage variation as a function of pH and NaOCl concentration, in Batch B.

Table S3. ANOVA for the Batch A of experiments regarding the chlorination response surface, using a Box-Behnken approach.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1.122E+04	9	1.247E+03	49.780	0		
	Error	425.841	17	25.049				
	Adjusted total	1.165E+04	26	448.019				
Factors	Intercept	5.155E+03	1	5.155E+03	205.798	0	41.453	2.890
	[NaOCl] /mg/L (A)	3.189E+03	1	3.189E+03	127.325	0	-14.119	1.251
	pH (B)	6.022E+03	1	6.022E+03	240.410	0	19.401	1.251
	T /°C (C)	516.539	1	516.539	20.621	0.0003	-1.136	0.250
	AB	203.112	1	203.112	8.108	0.0111	-3.101	1.089
	AC	58.916	1	58.916	2.352	0.1435	-1.670	1.089
	BC	125.770	1	125.770	5.021	0.0387	-2.440	1.089
	AA	26.235	1	26.235	1.047	0.3205	-1.326	1.296
	BB	879.297	1	879.297	35.102	0	7.679	1.296
	CC	2.774	1	2.774	0.111	0.7434	0.431	1.296
Model check	Main	9.728E+03	3	3.243E+03				
	Int	387.797	3	129.266	5.160	0.0102		
	Int + Sqr	1.107E+03	3	368.911	14.727	0.0001		
	Squ	1.107E+03	3	368.911	14.727	0.0001		
	Error	425.841	17	25.049				
Lack of fit	Lack of fit	193.198	3	64.399	3.875	0.0329		
	Pure error	232.643	14	16.617				
	Total error	425.841	17	25.049				

Table S4. ANOVA for the Batch A of experiments regarding the effective degradation response surface, using a Box-Behnken approach.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1.129E+04	9	1.254E+03	43.734	0		
	Error	487.415	17	28.671				
	Adjusted total	1.177E+04	26	452.794				
Factors	Intercept	1.028E+04	1	1.028E+04	358.654	0	58.547	3.091
	[NaOCl] /mg/L (A)	3.546E+03	1	3.546E+03	123.674	0	14.887	1.339
	pH (B)	5.713E+03	1	5.713E+03	199.247	0	-18.896	1.339
	T /°C (C)	579.124	1	579.124	20.199	0.0003	1.203	0.268
	AB	129.847	1	129.847	4.529	0.0483	2.479	1.165
	AC	61.716	1	61.716	2.153	0.1606	1.709	1.165
	BC	125.770	1	125.770	4.387	0.0515	2.440	1.165
	AA	5.887	1	5.887	0.205	0.6562	0.628	1.387
	BB	946.142	1	946.142	32.999	0	-7.966	1.387
	CC	0.312	1	0.312	1.090E-02	0.9181	-0.145	1.387
Model check	Main	9.838E+03	3	3.279E+03				
	Int	317.332	3	105.777	3.689	0.0327		
	Int + Sqr	1.130E+03	3	376.720	13.139	0.0001		
	Squ	1.130E+03	3	376.719	13.139	0.0001		
	Error	487.415	17	28.671				
Lack of fit	Lack of fit	250.377	3	83.459	4.929	0.0153		
	Pure error	237.038	14	16.931				
	Total error	487.415	17	28.671				

Table S5. ANOVA for the Batch B of experiments regarding the chlorination response surface, using a Box-Behnken approach.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1.689E+04	14	1.206E+03	33.758	0		
	Error	1.286E+03	36	35.729				
	Adjusted total	1.817E+04	50	363.448				
Factors	Intercept	7.050E+03	1	7.050E+03	197.316	0	48.477	3.451
	[NaOCl] /mg/L (A)	5.608E+03	1	5.608E+03	156.969	0	-15.287	1.220
	pH (B)	7.484E+03	1	7.484E+03	209.463	0	17.659	1.220
	T /°C (C)	624.341	1	624.341	17.474	0.0002	-1.020	0.244
	[DOM] /mg/L (D)	64.682	1	64.682	1.810	0.1869	1.642	1.220
	AB	171.403	1	171.403	4.797	0.0351	-2.222	1.014
	AC	659.027	1	659.027	18.445	0.0001	-4.357	1.014
	AD	31.760	1	31.760	0.889	0.3521	-0.956	1.014
	BC	199.900	1	199.900	5.595	0.0235	-2.399	1.014
	BD	1.088	1	1.088	3.045E-02	0.8625	0.177	1.014
	CD	9.052	1	9.052	0.253	0.6178	0.511	1.014
	AA	45.640	1	45.640	1.277	0.2659	-1.193	1.056
	BB	802.694	1	802.694	22.466	0	5.004	1.056
	CC	107.219	1	107.219	3.001	0.0918	-1.829	1.056
	DD	2.152	1	2.152	6.022E-02	0.8075	-0.259	1.056
Model check	Main	1.378E+04	4	3.445E+03				
	Int	1.072E+03	6	178.705	5.002	0.0008		
	Int + Sqr	2.033E+03	4	508.145	14.222	0		
	Sqr	2.033E+04	4	508.145	14.222	0		
	Error	1.286E+03	36	35.729				
Lack of fit	Lack of fit	759.415	10	75.942	3.748	0.0032		
	Pure error	526.837	26	20.263				
	Total error	1.29E+03	36	35.729				

Table S6. ANOVA for the Batch B of experiments regarding the effective degradation response surface, using a Box-Behnken approach.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1.686E+04	14	1.204E+03	35.146	0		
	Error	1.234E+03	36	34.264				
	Adjusted total	1.809E+04	50	361.860				
Factors	Intercept	7.964E+03	1	7.964E+03	232.428	0	51.523	3.380
	[NaOCl] /mg/L (A)	6.076E+03	1	6.076E+03	177.319	0	15.911	1.195
	pH (B)	6.930E+03	1	6.930E+03	202.258	0	-16.993	1.195
	T /°C (C)	671.512	1	671.512	19.598	0.0001	1.058	0.239
	[DOM] /mg/L (D)	84.150	1	84.150	2.456	0.1258	-1.873	1.195
	AB	88.379	1	88.379	2.579	0.1170	1.595	0.993
	AC	579.191	1	579.191	16.904	0.0002	4.084	0.993
	AD	31.760	1	31.760	0.927	0.3421	0.956	0.993
	BC	199.900	1	199.900	5.834	0.0209	2.399	0.993
	BD	0.839	1	0.839	2.447E-02	0.8766	0.155	0.993
	CD	9.052	1	9.052	0.264	0.6104	-0.511	0.993
	AA	28.871	1	28.871	0.843	0.3648	0.949	1.034
	BB	893.065	1	893.065	26.064	0	-5.279	1.034
	CC	115.461	1	115.461	3.370	0.0747	1.898	1.034
	DD	2.848	1	2.848	8.312E-02	0.7748	0.298	1.034
Model check	Main	1.376E+04	4	3.440E+03				
	Int	909.120	6	151.520	4.422	0.0019		
	Int + Sqr	2.189E+03	4	547.198	15.970	0		
	Sqr	2.189E+03	4	547.198	15.970	0		
	Error	1.234E+03	36	34.264				
Lack of fit	Lack of fit	706.494	10	70.649	3.485	0.0051		
	Pure error	527.021	26	20.270				
	Total error	1.234E+03	36	34.264				

2.3. The UV filter benzophenone-8 (BP8)

This study and all the work involved with it, was entirely carried out and fulfilled by the author, Albano Joel Moreira Santos, with supervision, guidance and revision being provided by Professor Dr. Joaquim Carlos Gomes Esteves da Silva.

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Environmental fate and behaviour of benzophenone-8 in aqueous solution

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Abstract

The fate and behaviour of 2-hydroxy-4-methoxyphenyl)-(2-hydroxyphenyl)-methanone, ordinarily known as benzophenone-8 (BP8), in aqueous solution, is thoroughly addressed in this study. The reactions were followed by means of high-performance liquid chromatography coupled with ultraviolet, diode-array detection (HPLC-UV-DAD). Potential by-products of degradation were analysed and determined by liquid chromatography coupled with diode-array detection and mass spectrometry (LC-DAD-MS). BP8 displayed very significant reactivity towards free available chlorine, following a pseudo-first order kinetic profile with rate constant $k_{\text{obs}} = 0.0036 \pm 0.0002 \text{ s}^{-1}$ and a half-life period of $t_{1/2} = 194.12 \pm 7.90$ seconds. Disinfection-induced degradation yielded only monochlorinated and dichlorinated disinfection by-products (DBPs). With or without dissolved organic matter (DOM) in solution, the pH and chlorine concentration were the only influential variables on degradation. Photo-induced degradation was assessed by means of exposure to artificial solar radiation. The compound proved to be extremely photo-stable, with a kinetic rate constant of $k = 0.00083 \pm 0.00002 \text{ min}^{-1}$ and a half-life period of $t_{1/2} = 835.12 \pm 20.12$ minutes. Exposure to artificial solar radiation did not generate any meaningful alterations in the UV spectrum of BP8. There are indications of the possible formation of a photo-isomer, but it's extremely low significance and intensity, even after pre-concentration, deemed it negligible.

Keywords

UV filters; 2-hydroxy-4-methoxyphenyl)-(2-hydroxyphenyl)-methanone; benzophenone-8; BP8; emerging pollutants; EPs; disinfection-induced degradation; photo-induced degradation; disinfection by-products; DBPs.

1. Introduction

Ultraviolet (UV) filters are part of a new class of organic compounds entitled emerging pollutants (EPs), a class which has gathered significant attention due to concerns regarding their features and pathways of inclusion into the aquatic environment. EPs encompass a substantial number of compounds of different families or groups, such as drugs of abuse, flame retardants, gasoline additives, personal-care products, pharmaceuticals, or even new classes such as nanomaterials. By definition, these compounds have not been thoroughly approached or studied in regards to their aquatic, environmental fate and behaviour, are believed to display potential eco-toxicological effects and are not yet constrained by water quality legislation [1]. In general, these compounds are structurally comparable to priority organic pollutants (POPs), since they typically display benzenic rings associated with one or more unsaturated side-chains, as well as prominent tendency towards environmental persistence and bioaccumulation [2-4].

The concerns about these compounds converge on their reactivity in aqueous solution and their toxicological features [1, 2, 5]. UV filters are associated with several potential toxicological effects [3], but they are also known to degrade significantly in aqueous solution and when into contact with active chlorine [6], leading to the formation of disinfection by-products (DBPs), which is a group of organic halogenated pollutants likewise linked to additional and potentially disturbing eco-toxicological characteristics [1].

The paths of incorporation of EPs into the aquatic environmental compartments, depend heavily on their precedent application or use, and are considered quite complex [1, 2]. On the other hand, EP environmental levels, and UV filters in particular, are often determined as vestigial in natural samples [1, 2]. Subsequently, the argument may be made that these compounds, despite being found prevalently in superficial natural waters, sediments, soils and even fish, do not actually reach toxicological effect concentrations. However, their physical-chemical properties may point to a different scenario, since they present significant lipophilicity, resistance to biodegradation and propensity towards accumulation in the environment and food chain. In this context, actual effect concentrations may in fact be reached, leading also to toxicity enhancement of other compounds found in solution [2, 3].

The most common or relevant toxicological implications of UV filter exposure in particular, are of hormonal nature, with many compounds presenting multiple hormonal or anti-hormonal activity, while half-maximum effect concentrations are considered comparable to other well-known hormonally-active pollutants [3]. Since UV filters and EPs in general are of synthetic origin, waste water treatment procedures are of vital importance on how to deal with these pollutants, and concerns exist over their efficiency on eliminating the more polar compounds from the water courses [7, 8].

As far as the degradation of UV filters is concerned, there are two important sources to emphasize: disinfection-induced degradation; and photo-induced degradation. A few studies exist on the degradation of these compounds [8-13], but these are either focused on the same small number of filters, namely those relatively considered more popular in some commercial sunscreen formulations, or they regard solely one degradation scenario, in particular the disinfection-induced degradation, which is fundamentally achieved worldwide by the use of chlorine or chlorine-related water disinfecting agents. One of the problems highlighted by the analysis of these publications, besides the issue with addressing only one degradation source, is the fact that authors seldom focus on other common UV filters, out of a comprehensive list of more than 40 compounds currently regulated and used by pharmaceutical and cosmetic companies in their formulations [4, 14].

The purpose of this work, is to extensively and thoroughly investigate the degradation reactions of (2-hydroxy-4-methoxyphenyl)-(2-hydroxyphenyl)-methanone, trivially known as benzophenone-8 (BP8) (see Figure 1 for molecular structure, and Table 1 for physical-chemical properties), in aqueous solution, and both in exposure to active free chlorine and artificial solar radiation. More specifically, the points covered are as follows:

- Determination of reaction kinetics and degradation rates, in both degradation scenarios, by using high-performance liquid chromatography coupled with ultraviolet diode-array detection (HPLC-UV-DAD), in order to accurately follow the reactions with direct sample injection and analysis;
- Influence of external variables on degradation [such as pH, water temperature, active chlorine or dissolved organic matter (DOM) concentrations], will be ascertained experimentally, by means of an elaborate design formulation, with subsequent statistical treatment and analysis, in order to highlight underlining trends;

- Identification of potential degradation by-products, from active chlorine-induced and artificial radiation-induced degradation, will be carried out by using liquid chromatography coupled with ultraviolet, diode-array detection and mass spectrometry (LC-DAD-MS);
 - To enhance the detection and identification capabilities of the procedure and ensure the reliability of the results, a solid-phase extraction (SPE) protocol was always applied to the studies;
- Alterations to BP8's UV absorption capabilities, along with exposure to artificial solar radiation, will be studied by spectrophotometric analysis;

So far, only one study has been presented regarding BP8 [12], and it was solely focused on the determination of the kinetic features of its disinfection-induced degradation. The comparison and discussion about the differences between this study and [12] will be approached in Section 3.1.1. The disinfection context in the study, will be ensured by the addition of free, active chlorine, as it is the vastly most popular water disinfecting agent used worldwide. Subsequently, the present work aims at providing a frame of reference and analogy in that context, but mostly, it intends to fill the gaps on the remaining aspects of the compound's fate and behaviour when in aqueous solution. Are there any stable DBPs formed throughout the disinfection-induced reactions? If so, how many DBPs are produced and detected? Are their levels significant or merely considered trace levels? How does BP8 disinfection-induced degradation evolve, considering changes in the water temperature, pH, concentration of active chlorine in solution or even DOM concentration? DOM is important in the context of water disinfection, because many natural organic compounds are found in superficial water bodies (such as swimming pools and lakes), namely from human activities or behaviours, and are known to actively compete with synthetic organic compounds for the available free chlorine in solution. Is BP8 photo-stable under exposure to UV radiation? Are there any by-products generated through exposure to UV radiation? These are the questions this work proposes to answer, and therefore, provide a comprehensive perspective on the fate and behaviour of BP8 in the aquatic environment.

2. Materials and methods

2.1. Reagents

The UV filter BP8 (CAS registry number 131-53-3; and molecular formula $C_{14}H_{12}O_4$) was purchased as an analytical grade standard from Sigma-Aldrich, Merck, Darmstadt, Germany. A reagent grade sodium hypochlorite (NaOCl) solution, with 3.5% active chlorine labelled content, was purchased from VWR Chemicals, Radnor, PA, USA. From this commercial solution, a stock solution of 432 mg/L in active chlorine content, was prepared and permanently stored at 4°C and removed only upon use. Active chlorine content was evaluated by means of the standard iodometric titration method. The compound L-ascorbic acid (at 99%) was purchased from Merck, Darmstadt, Germany. Deionised water (conductivity < 0.1 μ S/cm) was the only water source in the entire study. The pH was always regulated and adjusted with the addition of ordinary standard solutions of either hydrochloric acid (HCl) or sodium hydroxide (NaOH). Pre-concentration of the entire reaction solution volumes, necessary for the analysis and identification of by-products, was carried out by solid-phase extraction (SPE), using LiChrolut EN 200 mg, 6 mL standard cartridges from Merck, Darmstadt, Germany. All organic solvents used for pre-concentration or chromatographic purposes, namely methanol, acetonitrile or dichloromethane, were of liquid chromatographic grade and purchased from Merck, Darmstadt, Germany. Dissolved organic matter (DOM) was obtained from pinewood soil [15-17].

2.2. Chromatographic conditions

2.2.1. LC-UV-DAD

In order to study the degradation reactions of BP8, high performance liquid chromatography coupled with ultraviolet, diode-array detection (HPLC-UV-DAD) was implemented as the instrumental basis of this work. This system was overall composed of an isocratic pump (Hewlett–Packard 1100 Series, Böblingen, Germany), a manual sample injection valve with a 20 μ L loop (Rheodyne 7725i, Rohnert Park, USA), a silica-based C18 reverse phase column [Dionex Acclaim™ 120 C18, particle size 3 μ m, pore diameter 175 Å (4.6 x 150 mm), Thermo Fisher Scientific, Sunnyvale, CA, USA], and a photo-diode-array detector (UV 6000LP with a 50 mm LighPipe flow cell, Thermo Fisher Scientific, Sunnyvale, CA, USA). In order to successfully optimize and validate the analytical method, previous knowledge and experience gathered with the LC system and this class of compounds [6], were of paramount importance. Optimization of the method, included the study of several different solvents, solvent mixtures, solvent ratios and flow rates. The conditions that yielded the best overall performance results, namely regarding

accuracy, precision, peak intensity, peak shape, peak definition, result repeatability and ultimately, system linearity, were as follows: the mobile phase used to investigate the degradation reactions, was a mixture of liquid chromatography-grade acetonitrile and deionised water (80:20, v:v), at a constant 0.60 mL/min flow rate. Before being used, mobile phases were always filtered by pressure through a borosilicate glass funnel (pore size 4-8 microns). UV absorbances were monitored and recorded at a total scan mode (200-600 nm), and the entire system was controlled and monitored with the Xcalibur 1.4 SR software program.

2.2.2. LC-DAD-MS

The analysis and determination of all the potential by-products of the degradation reactions, were carried out by liquid chromatography coupled with diode-array detection and mass spectrometry (LC-DAD-MS). The system was composed of a high performance liquid chromatography pump (Finnigan Surveyor LC Pump Plus), an auto-sampler (Finnigan Surveyor Auto-sampler Plus) and a photo-diode array detector equipped with a LightPipe flow cell (Finnigan Surveyor PDA Plus Detector) (all instrumentation was purchased from Thermo Electron Corporation, Waltham, USA), coupled with the same reverse phase silica-based column presented in the previous Section [Dionex Acclaim™ 120 C18, particle size 3 µm, pore diameter 175 Å (4.6 x 150 mm), Thermo Fisher Scientific, Sunnyvale, CA, USA]. The mass spectrometer was a Finnigan LCQ Deca XP Max (Thermo Electron Corporation, Waltham, USA) coupled to the LC system and equipped with an electrospray interface as ionization source and a quadrupole ion trap for MSⁿ experiments. Details on the successful identification of by-products will be discriminated and addressed in the following Sections, but the system was ultimately operated in negative ion mode between $m/z = 150$ and 1000, with the following conditions: spray voltage, 5.0 kV; capillary voltage, 15 V; and capillary temperature, 300°C. The device was programmed to perform a full mass scan first, and then a fragmentation analysis (MS/MS) of the most intense ion each peak. The analytical method was essentially the same as the one described in the previous section, as was the column used. The mobile phase was the same used in the HPLC-UV-DAD analysis, although ammonium acetate was added as a mobile phase modifier (at 0.005 mol/L) [8], in order to promote the ionization of the compounds of interest and therefore, their detection and identification. The flow rate was the same as in the previous Section, 0.60 mL/min, and the mobile phase was also always filtered in the same conditions, prior to

use. The software program Xcalibur 1.4 SR was used to control and monitor the entire system and experiments.

2.2.3. Performance of the analytical method

The analytical method was designed and evaluated according to the ICH standard guidelines for the validation of analytical procedures [18]. Validation was accomplished on the basis of accuracy, precision, linearity and detection limit. Calibration of the method was accomplished with the analysis of eleven solutions between the range 0.02500 - 25.00 mg/L. Three trials were carried out and analysed for each solution. Calibration curves were completely rectilinear throughout the aforementioned calibration range and correlation coefficients obtained were all higher than $R^2 = 0.997$, yielding a detection limit of 0.47 $\mu\text{g/L}$.

2.3. Disinfection-induced degradation

2.3.1. Determination of reaction kinetics

All the experiments described in this Section were carried out in aqueous solution, at neutral pH, room temperature, in the dark and with constant stirring. These conditions were designed to mimic, as much as possible, a swimming pool aquatic environment.

A stock solution of BP8 was first prepared, by dissolving 100 mg of compound in 100 mL of absolute ethanol (final concentration 1000 mg/L or 4.094×10^{-3} mol/L). Every experiment conducted subsequently, was spiked with an adequate volume of this solution, in order to match the required final reaction concentration.

In more detail, degradation reactions were conducted in 250 mL borosilicate glass beakers, with deionized water as the medium, and BP8 at 0.5000 mg/L (2.047×10^{-6} mol/L) and NaOCl at 5.002 mg/L (6.716×10^{-5} mol/L), in a final aqueous solution of 100 mL volume. This experimental scenario will ensure that the variation of NaOCl levels along with time can remain inconsequential, in principle. Before the start of the reaction, that is, before the addition of NaOCl to the solution, an aliquot of the aqueous solution containing solely BP8 was always analysed by direct injection in the HPLC-UV-DAD system, providing the necessary chromatographic record of the initial levels of the filter. Subsequently, NaOCl would be added to the solution, the water temperature was recorded and monitored ($T = 22.2 \pm 0.7^\circ\text{C}$), the pH was adjusted and monitored ($\text{pH} = 6.2 \pm 0.5$), if necessary, with the addition of a few drops of ordinary standard solutions of

HCl or NaOH, and the reaction timer initiated. The experiments took place in a total time-frame of 240 seconds, and an aliquot of the solution was always removed at each period of 20 seconds. In order to stop the degradation reaction, the residual active chlorine was removed with the addition of 20 μ L of a standard ascorbic acid (AA) solution of approximately 65 g/L, and the samples were stored at 4°C until the moment of analysis by direct injection in the HPLC-UV-DAD system.

2.3.2. Determination of disinfection by-products (DBPs)

In order to detect and identify DBPs of the disinfection-induced degradation reactions of BP8 with active chlorine, the experimental conditions discriminated in the previous Section were reproduced with a different emphasis.

In this case, the reaction was initiated and then left undisturbed for about 15 minutes, a period after which, the entire solution volume was pre-concentrated by means of SPE (by using LiChrolut EN 200 mg, 3 mL cartridges from Merck, Darmstadt, Germany). Prior to the pre-concentration step itself, the cartridges were first conditioned with 3 mL of methanol and then 3 mL of deionized water. Next, the entire mixture was made to pass through the cartridge, with the help of a peristaltic pump (Minipuls® 2, from Gibson, Inc., Middleton, WI, USA). Subsequently, the cartridges were washed with an additional portion of 3 mL of deionized water, and then the compounds of interest were eluted with 3 mL of dichloromethane. The organic solvent was evaporated with a gentle flow of nitrogen gas, and the remaining residue diluted in 500 μ L of methanol. This final solution was then directly injected and analysed in the LC-DAD-MS system.

The analysis by LC-DAD-MS was performed in negative mode, and the mobile phase used was similar to the one used in the previous studies, although it was modified with the addition of ammonium acetate at 0.005 mol/L [8] [acetonitrile-deionized water-ammonium acetate (80-19.5-0.5)], in order to promote the successful identification of the compounds by mass spectrometry.

2.3.3. External influences on disinfection-induced degradation

In aqueous solution, disinfection-induced degradation of organic compounds may depend on or be influenced by several external variables, such as active chlorine levels in solution, water temperature, pH and DOM levels.

To study the influence of these variables on the degradation rates of BP8, experimental design formulations were built and the results then investigated by statistical analysis of variance (ANOVA). This portion of the work was enabled by and processed with The Unscramber® v9.2 software, from CAMO Process AS, Oslo, Norway.

More specifically, two Box-Behnken sets of experiments were designed (Set A and B; see Figure 2), with corresponding response surface methodology. One set did not include DOM in solution (Set A), while the other (Set B) included DOM as an ordinary design variable. The details on the global study are included in Figure 2, with additional information on the experimental conditions of both sets, as well as statistical analysis and corresponding validation of the models, included in Section 2 of the supplementary material provided. Functionally, experiments were performed in the same manner as described in Section 2.3.1, with all the experimental conditions respecting the specifications detailed in Tables S1 and S2 of Section 2 of supplementary material. After a pre-defined period of reaction, an aliquot was removed from the reactor, its active chlorine content quenched with 20 µL of a standard 65 g/L AA solution, and then analysed by direct injection into the HPLC-UV-DAD system.

2.4. Photo-induced degradation

Despite being used or applied topically on human skin, as part of sunscreen lotion formulations, UV filters are known to pass into the water course through numerous and complex ways and paths [1, 2]. Consequently, and in aqueous solution, just as exposure to disinfecting agents affects the structure of UV filters, leading to their degradation and formation of DBPs, exposure to UV radiation also induces similar processes [19-21].

2.4.1. Determination of reaction kinetics

Exposure of the experimental solutions to solar radiation, was carried out by artificial means, using a solar radiation simulator (Newport Oriel 96000 full spectrum Solar Simulator, with an ozone-free 150W xenon lamp, with Air Mass filters 1.5 Global 81094, producing a 33 mm diameter collimated beam) with a liquid light guide, the tip of which was always positioned about 1 centimetre above the water surface. The experimental conditions were slightly similar to those described in previous Sections. Conventional 250 mL borosilicate beakers, containing 100 mL of aqueous solutions with BP8 at 0.5000 mg/L (2.047×10^{-6} mol/L) and under constant stirring, were exposed to artificial solar

radiation during a reaction time-frame of 360 minutes in total. At each period of 60 minutes, an aliquot was always removed and analysed, first, in the UV-visible spectrophotometer, to evaluate potential changes in the UV absorption spectra of the compound, and second, by direct injection in the HPLC-UV-DAD system, in order to follow the degradation reaction and determine its kinetic features.

2.4.2. Determination of photo-induced by-products

Analysis of the degradation reactions from the previous Section, was crucial in order to determine or identify potential by-products being formed and detected in the chromatograms.

The reactions from the previous Section were exactly reproduced and allowed to run its course, for a time-frame of 300-360 minutes. After this period of artificial irradiation, the entire solution volume was pre-concentrated by means of SPE, in the exact same manner detailed in Section 2.3.2, and the resulting extract was analysed by direct injection in the LC-DAD-MS system.

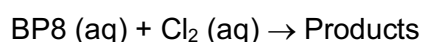
3. Results and discussion

3.1. Disinfection-induced degradation

3.1.1. Determination of reaction kinetics

As it was mentioned in Section 2.3.1, disinfection-induced degradation was studied in aqueous solution, with BP8 levels at 0.5000 mg/L (2.047×10^{-6} mol/L), active free chlorine levels at 5.002 mg/L (6.716×10^{-5} mol/L), at neutral pH ($\text{pH} = 6.2 \pm 0.5$), room temperature ($T = 22.2 \pm 0.7^\circ\text{C}$), in the dark and under constant stirring.

Oxidation of organic and inorganic compounds in aqueous solution and with exposure to free active chlorine, usually follows a global second order reaction rate, as stated in Deborde's and Von Gunten's extensive revision work [23]. More specifically, the reaction will be first order in regards to the organic or inorganic compound and first order in relation to active chlorine. In this case, the degradation reaction between BP8 and the total amount of active chlorine in solution ($[\text{Cl}_2]_t = [\text{HOCl}] + [\text{ClO}^-]$) can be defined as:



With the corresponding kinetic law being:

$$v = -(\Delta[\text{BP8}]/\Delta t) = k_{\text{app}}[\text{BP8}][\text{Cl}_2]_t$$

Where $[\text{BP8}]$ represents the concentration of the UV filter BP8, k_{app} is the apparent global second order rate constant, and $[\text{Cl}_2]_t$ is the total concentration of active free chlorine species in solution (HOCl and ClO^-), the predominance of which is pH-dependent. Under pseudo-first order conditions, however, the kinetic law can be simplified into the following:

$$v = -(\Delta[\text{BP8}]/\Delta t) = k_{\text{obs}}[\text{BP8}]$$

Where k_{obs} is the observed pseudo-first order rate constant of the degradation reaction ($k_{\text{obs}} = k_{\text{app}}[\text{Cl}_2]_t$).

Figure 3 presents the exponential plot of the mean values of the concentration of BP8 as a function of reaction time. From the corresponding experimental data and plot, we have:

$$[\text{BP8}]/[\text{BP8}]_0 = 0.919479e^{-0.003571t}$$

The linear regression allowed the determination of the observed pseudo-first order degradation rate ($k_{\text{obs}} = 0.0036 \pm 0.0002 \text{ s}^{-1}$) and also the corresponding half-life period ($t_{1/2} = 194.12 \pm 7.90$ seconds). Therefore, results demonstrate that BP8 degrades quite substantially and rapidly over the course of only a few minutes of reaction time, and at neutral pH, reaching 40% of the initial levels at the end of 4 minutes. In the only existing study on BP8 [12], the authors only provided a plot of the observed pseudo-first order degradation rate of the compound as a function of free available chlorine, for $9 \leq \text{pH} \leq 11$, and nonetheless the values are equivalent to those reported in this work. For analogy purposes, BP3, another benzophenone UV filter studied [12], the plot presented was limited to $6 \leq \text{pH} \leq 9$, and the values are also comparable, even considering the fact that we are referring to homologous compounds. Additionally, what was indeed evident in these different studies, was that the reactivity of BP8 (and benzophenone filters in general) seems to increase with the increase in pH, which may indicate that the ionic form of the compound might predominate in solution, over its neutral form. Figure 3 presents the exponential plot of the relative amounts of BP8 as a function of reaction time at neutral pH, and the values are much lower than those reported for $\text{pH} = 8$ [12]. A similar trend was also found for the related compound BP4 [8], with its reactivity

increasing significantly as the pH increased from 6 to 8. Half-life periods were not reported or referred by the authors [8, 12], for comparison purposes.

3.1.2. Determination of disinfection by-products (DBPs)

The degradation reaction and the corresponding originated DBPs, are presented in Figure 4, which includes a montage of the LC-DAD-MS total-ion current (TIC) chromatogram along with the mass spectra of the identified compounds. Additional data on the identification of the peaks is provided in Table 2, including retention times, molecular ions, peak fragments, corresponding predominance, and proposed molecular formulas. Figure 5 (included in supplementary material) presents an analogy between the UV spectra of the different identified compounds, that is, the parental UV filter BP8 and its mono (Cl-BP8) and dichlorinated (Cl₂-BP8) structures. Figure 6 represents the overall sequential degradation reaction, leading to the formation of the aforementioned DBPs, along with the identification of the most probable sites for the electrophilic substitution of active chlorine onto the molecule. Fragmentation paths and molecular formulas of the most prominent ion fragments, are presented in Section 1 of the supplementary material provided, specifically in Figures S1-S9. All the detailed information regarding the identification of the compounds, is also sequentially compiled in the supplementary material, including the mass spectra of the peaks and their fragmentations.

The analysis of the degradation reaction by LC-DAD-MS led to the successful detection and identification of two DBPs, the monochlorinated (Cl-BP8) and dichlorinated (Cl₂-BP8) structures of BP8, indicated in the chromatogram of Figure 4 at retention times 6.77 and 7.61 minutes, respectively. The analysis of the first peak at retention time 6.01 minutes, led to the expected confirmation of the original compound, BP8. The chlorination of aromatic compounds takes place essentially by electrophilic substitution of the chlorine atom on benzenic rings, namely those displaying particular functional groups that have an activating role towards that substitution, as Deborde and Von Gunten have highlighted [23]. Functional groups such as hydroxile (-OH) and methoxy (-OCH₃), both present in the same benzenic ring of the BP8 structure, are amongst the strongest *ortho* and *para*-activating groups. In other words, this means that the electrophilic substitution of active chlorine onto the compound, will most likely take place on the benzenic ring displaying both these groups. Considering the compound's structure and the position of these groups on the molecule, the active chlorine will attack preferably at the *ortho* and *para* positions in regards to the -OH group, or at both the

ortho positions in regards to the -OCH₃ group, as is demonstrated in Figure 6. The detection and identification of the DBPs through LC-DAD-MS analysis, was fairly straightforward as all compounds responded very well and were easily detected by MS. Fragmentation details are presented in Table 2 and proposed fragmentation paths and subsequent fragment ions are also reported in Section 1 of the supplementary material provided, with molecular structures being given for the most prevalent ions.

3.1.3. Influences of external variables on degradation

There are several considered external variables that characterize and define superficial, aquatic, environmental compartments (such as lakes, rivers or even swimming pools), namely water temperature, chlorine concentration, pH or DOM levels in solution. Subsequently, these will manipulate the fate and behaviour of synthetic organic pollutants in aqueous solution. This portion of the study is focused on determining whether or not these external variables carry a significant influence over the degradation rates of BP8, and if so, the extent of that influence. For this purpose, the work was conducted in the form of a Box-Behnken multivariate design formulation with response surface methodology.

Functionally, the study was divided in two models or sets, as detailed in Section 2.3.3 and Figure 2: Set A (without DOM in solution); and Set B (with DOM in solution). Additional information about this part of the work, for example, the experimental conditions and number of experiments, is included in Section 2 of the supplementary material provided. The analysis of variance (ANOVA) carried as well as the specifics on the statistical validation of the models [24] are also included.

As far as Set A is concerned, the following equation represents the quadratic response surface equation:

$$\hat{Y} = 35.573 + 6.018A + 2.958B - 0.008750C + 1.356AB + 4.146AC - 1.186BC - 4.154AA - 7.502BB - 4.544CC$$

Where A represents NaOCl concentration (in mg/L), B represents the pH and C represents water temperature (in °C). The remaining variables (AB, AC, BC, AA, BB and CC) are the quadratic interactions between them. Results show that the most influential variables in Set A, were NaOCl concentration ($\beta = 6.018 \pm 0.298$) and the pH ($\beta = 2.958 \pm 0.149$). Reactivity of BP8 towards active chlorine in aqueous solution, was already deemed as quite significant as rapid, so these results were expected. As is shown by the

response surface of Set A (see [Figure 7](#)), obtained as a function of the interaction NaOCl x pH, degradation increases with the amount of active chlorine in solution and the increase of pH. Hypochlorous acid (HOCl) is widely regarded as the most efficient chlorine disinfecting agent used in superficial water bodies, prevalent at neutral to acidic pH values [23]. Higher degradation rates found at higher pH values, though, means that BP8 reacts more significantly with hypochloride anion (ClO^-), not considered a very efficient chlorine disinfecting agent and prevailing at alkaline pH values [23]. Data then supports the strong possibility of the ionic form of BP8 dominating over the non-ionic form of the compound, when in aqueous solution.

Regarding Set B, the quadratic response surface equation is as follows:

$$\hat{Y} = 24.803 + 1.684A - 0.273B + 1.953C - 0.08850D - 0.385AB - 1.557AC + 1.019AD - 0.247BC + 0.893BD + 0.259CD - 1.524AA - 2.851BB - 2.439CC - 3.462DD$$

With A representing NaOCl concentration (in mg/L), B being DOM concentration (in mg/L), C as the pH and D as the water temperature (in °C). The remaining terms (AB, AC, AD, BC, BD, CD, AA, BB, CC, and DD) represent the quadratic interactions between those variables. As was the case with Set A, data suggests that the most influential variables in Set B were also NaOCl concentration ($\beta = 1.684 \pm 0.450$) and the pH ($\beta = 1.953 \pm 0.225$). The response surface for Set B, is presented in [Figure 8](#). Results for Set B were somewhat similar to those from Set A, with NaOCl and pH emerging also as the most influential variables on degradation of BP8, in the presence of DOM. Moreover, data indicates that DOM little influence in the process, with one of the lowest beta coefficients of the response surface equation and a corresponding standard deviation almost as significant as the coefficient value itself ($\beta = -0.273 \pm 0.225$). Nonetheless, despite displaying very little influence in the process, when compared to the other variables, one must consider the negative sign of DOM's beta coefficient. Despite its failure to cause significant impact on degradation, this means that, in its own limited context, degradation seemingly decreases with the increase in DOM levels, which is to be expected. On the other hand, considering DOM's natural proclivity towards active chlorine in solution, it was expected to have a more compelling role, at least in lowering the degradation rates. In this regard, it is important to emphasize, however, in favour of DOM's role in hindering the degradation rates of BP8, that the values for the degradation percentages were found to be much lower when DOM was present in solution. Without DOM in solution (Set A), degradation values ranged from 7-39% (minimum-maximum), whereas with DOM included (Set B), values ranged from 7-26% (minimum-maximum),

as shown in Figures 7 and 8. In light of these numbers, one must conclude that DOM was indeed able to slightly compete with BP8 for the free available chlorine in solution, resulting in lower values for the maximum degradation rates.

3.2. Photo-induced degradation

3.2.1. Determination of reaction kinetics and photo-stability

Photo-induced degradation studies were carried out according to the experimental conditions outlined in Section 2.4.1. In 250 mL borosilicate beakers, 100 mL of deionized water were spiked with BP8 to reach a solution concentration of 0.5000 mg/L (2.047×10^{-6} mol/L), and placed under constant stirring. Photo-degradation was ensured through exposure to artificial solar radiation, using a solar radiation simulator. The device's details are also presented in Section 2.4. Prior to exposure to artificial radiation, the experimental solutions were always analysed in both the HPLC-UV-DAD and UV-visible spectrophotometer systems, in order to determine their initial conditions. In other words, to obtain a chromatographic record of the initial concentration of the compound and also a UV absorption spectrum profile of the solution before irradiation. After this step, the solutions were then exposed to artificial solar radiation for a total reaction period of 6 hours, with an aliquot being removed and analysed, in the HPLC-UV-DAD and spectrophotometer, at every hour period, in order to determine the photo-degradation kinetic features and possible UV absorption alterations throughout the experiment.

Figure 9 presents the exponential plot of the relative mean levels of compound as a function of irradiation time. The photo-degradation of BP8 followed a first-order kinetic profile, generating a rate constant of $0.00083 \pm 0.00002 \text{ min}^{-1}$ and a corresponding half-life period of 835.12 ± 20.12 minutes. There are no other photo-degradation studies of this nature carried out on BP8, for proper analogy purposes. Nonetheless, results show that the compound is extremely photo-stable, reaching just about 25% degradation after a period of 6 hours of artificial irradiation. As the compound's half-life period indicates, BP8 would reach a 50% degradation rate only after about 14 hours of irradiation. This constitutes remarkable photo-stability features, which might well be explained by the compound's molecular structure, rendering it significantly stable by very efficient electronic delocalization. Figure 10 highlights yet another aspect of this prominent photo-stability of BP8, by showing that there are little to no differences in their UV spectra absorption profiles before and after irradiation. In essence, the UV absorption capacity of the compound in aqueous solution, was found not to have been hindered in the least,

by exposing the solution to artificial solar radiation up to 300 minutes. Results demonstrate, therefore, that BP8 is extraordinarily photo-stable in aqueous solution, when exposed to artificial solar radiation.

3.2.2. Determination of potential photo-degradation by-products

After the photo-stability assessment of BP8 in aqueous solution, it was important to investigate whether or not the compound's photo-degradation reaction yielded any measurable amounts of by-products, such as photo-isomers [5]. The approach to this part of the study, was detailed in Section 2.4.2, and was also similar to the one from the analysis of DBPs.

According to the results, and despite there being some indications of a possible photo-isomer of BP8 in the SPE extracts obtained and analysed by LC-DAD-MS, the low intensity and significance of the peak did not allow for its unequivocal determination. This low significance of the peak was indeed very detrimental to the identification of this possible by-product, but additionally, the MS analysis indicated that the ion associated with BP8 ($m/z = 243$) was also not the most prominent or abundant molecular ion of the peak, which prevented the fragmentation of the intended mass, in the hopes of comparing the fragments to those of BP8 itself. Despite these indications, though, that which is certain is that this hypothetical photo-isomer of BP8 does not form in minimally significant amounts for it to be decisively identified, even after pre-concentration of the solution by SPE.

4. Conclusions and future challenges

The main purpose of this work, was to thoroughly ascertain the fate and behaviour of the UV filter BP8 in aqueous solution and conclude as to its degradation rates and profiles in both possible scenarios (disinfection-induced and photo-induced).

Regarding disinfection-induced degradation, results have shown that BP8 is extremely reactive towards free active chlorine, in neutral pH ($pH = 6.2 \pm 0.5$), room temperature ($T = 22.2 \pm 0.7^\circ C$) and in the dark. Data allowed the determination of the compound's degradation rate constant ($k_{obs} = 0.0036 \pm 0.0002 \text{ s}^{-1}$) and corresponding half-life period ($t_{1/2} = 194.12 \pm 7.90$ seconds), with BP8 reaching a 60% degradation rate at the end of each experiment, that is, 240 seconds. Merely two DBPs were detected and successfully determined by LC-DAD-MS throughout these reactions, namely the

mono and dichlorinated forms of BP8, with the electrophilic substitution of chlorine occurring rapidly and, most probably, at the ortho and para positions towards the -OH functional group (or at both ortho positions towards the -OCH₃ group). The most influential external variables on BP8 degradation, with or without DOM in solution, were found to be NaOCl concentration and pH. Higher degradation rates were obtained at higher concentrations of active chlorine in solution and at higher pH values, which indicates that the ionic form of the UV filter might predominate over its non-ionic form, when in aqueous medium. Given its competitive nature towards active chlorine, DOM was expected to be a very influential variable, but such was not found. DOM ended up behind NaOCl concentration and pH, as the most influential variables on degradation. However, even in this context, DOM was indeed found to have influence in the process, by decreasing the overall maximum degradation rates obtained in the studies. Set A (without DOM in solution) of experiments yielded maximum degradation rates of 39%, whilst Set B (with DOM in solution) of experiments originated maximum rates of 26%.

As far as the photo-induced degradation is concerned, BP8 was found to be extremely photo-stable, when exposed to artificial solar radiation, with a half-life period of $t_{1/2} = 835.12 \pm 20.12$ minutes and a degradation rate constant of $k = 0.00083 \pm 0.00002 \text{ min}^{-1}$. Results indicate that BP8 reached a maximum degradation rate of 25%, after 6 hours of artificial irradiation, and would only reach 50% degradation after 14 hours of exposure. Artificial irradiation did not lead to any meaningful alterations in the UV spectra of the compound and its absorption capacity. There are indications of the formation of a possible photo-isomer in the reaction, but it's extremely low intensity, even after pre-concentration, deems it fairly negligible.

Several questions were indicated in the Introduction of this work, and this study allowed the authors to reach an answer for each. [Figure 7](#) resumes these views and conclusions. But in essence, of all the dozens of UV filters currently approved and regulated by the most important legislative bodies, the crushing majority still remains unapproached and unstudied, as far as their fate and behaviour in the aquatic environment is concerned. BP8 was only studied once, in an interesting work [\[12\]](#), but focused solely on one perspective of the entire degradation scenario, leaving aside prominent aspects such as the formation of DBPs or even the external influences on degradation, as well as the important role of photo-degradation. This study was conducted to provide a meaningful context and analogy to that report, but especially to provide the most comprehensive evaluation on the fate and behaviour of an important

UV filter, and how it relates to the integrity of the aquatic environment, its natural and representative organisms as well as human health.

These aspects are crucial, in what concerns the challenges inherent to this particular class of compounds, which is the very low number of filters studied or approached. There are more than 40 filters currently approved and regulated worldwide, and merely a handful has ever been approached [6, 8-12, 25], with most of these studies dealing only with disinfection-induced degradation or the determination of DBPs. There are concerning reports on their potential eco-toxicological profiles, as well as their DBPs, but results remain inconclusive [2]. Reports on UV filter determination in superficial waters [2], are also limited, and hint at concentrations possibly too low to pose a threat to the environment, the aquatic organisms and human health. On the other hand, these compounds' physical-chemical properties indicate significant proclivity towards environmental persistence and bioaccumulation [2, 3]. Indeed, these compounds are being widely detected in all kinds of superficial waters [1], as well as in the food chain itself, namely in fish [1, 3], but also in human breast-milk [3, 26]. In other words, exposure to these chemicals is now hardly seasonal, during the months of Summer, as they seem to accumulate in the environment and food chain. Additionally, many other commercial products now include UV filters as primary components, besides the typical sunscreens, which may increase the concerns over long-term exposure and accumulation in the aquatic environments beyond the period of Summer.

Conclusive fate and behaviour evaluations, with disinfection and photo-induced degradation studies, are seldom carried out [6, 25]. The natural conclusion to this problem, is that more compounds must be studied, particularly since the works published indicate considerable variations in reactivity, degradation rates and DBP formation profiles. This step is essential, so that the fate and behaviour of these compounds may no longer remain generally obscure.

Acknowledgements

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(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf), and accessed on 21/06/2017.

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Figures

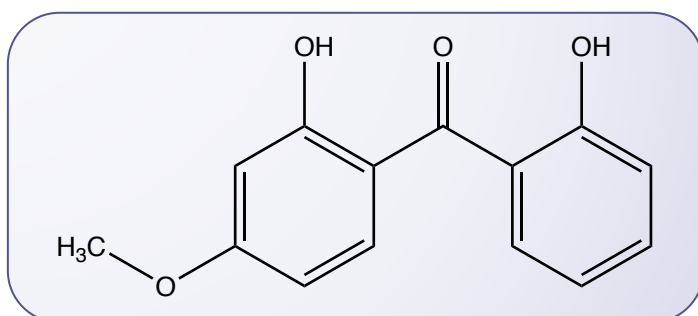


Figure 1. Molecular structure of (2-hydroxy-4-methoxyphenyl)-(2-hydroxyphenyl)-methanone, trivially known as benzophenone-8 (BP8).

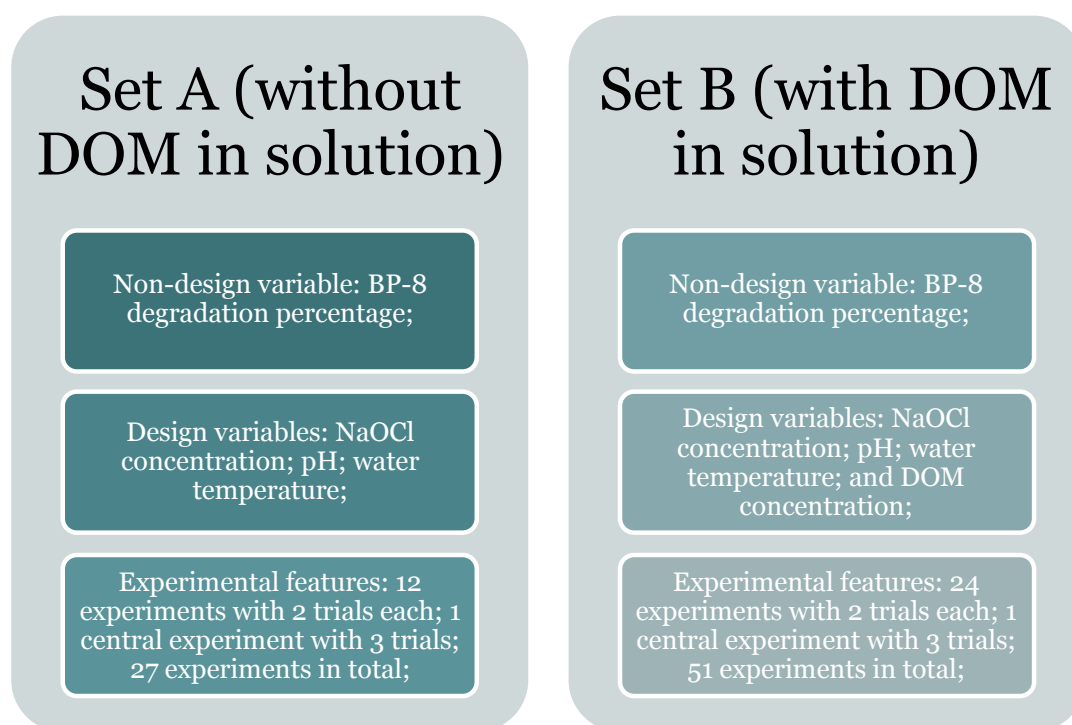


Figure 2. Experimental conditions of the Box-Behnken sets of experiments, to evaluate the influence of external variables on degradation of BP8.

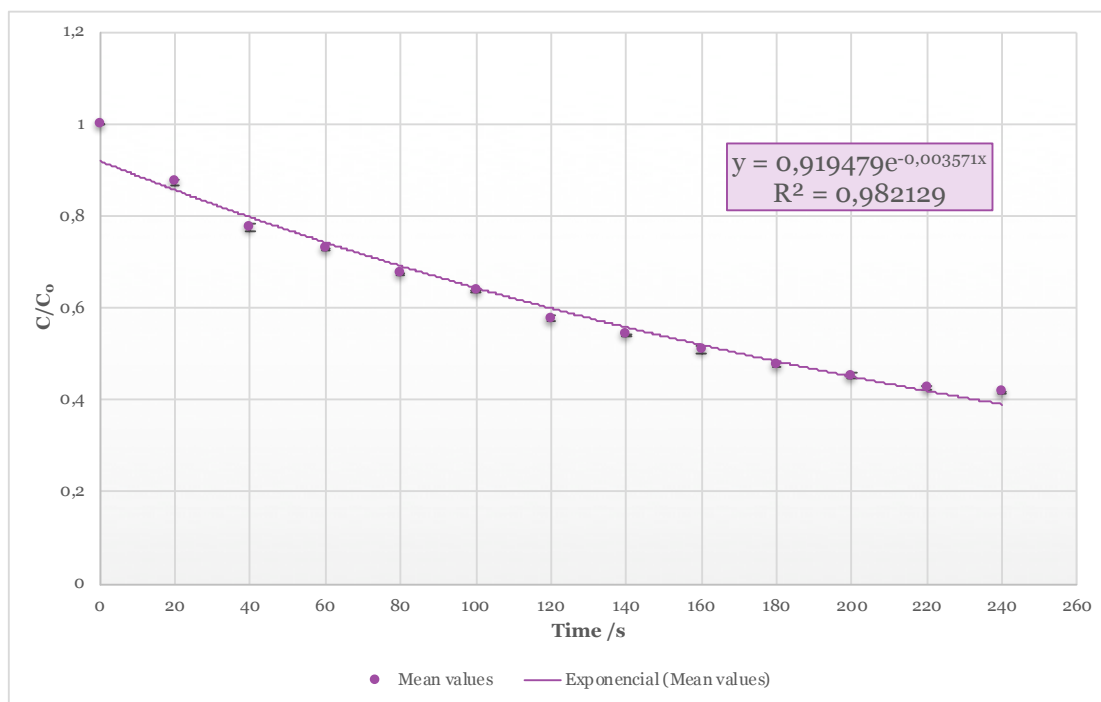


Figure 3. Exponential plot of BP8 concentration as a function of reaction time, for a disinfection-induced degradation reaction.

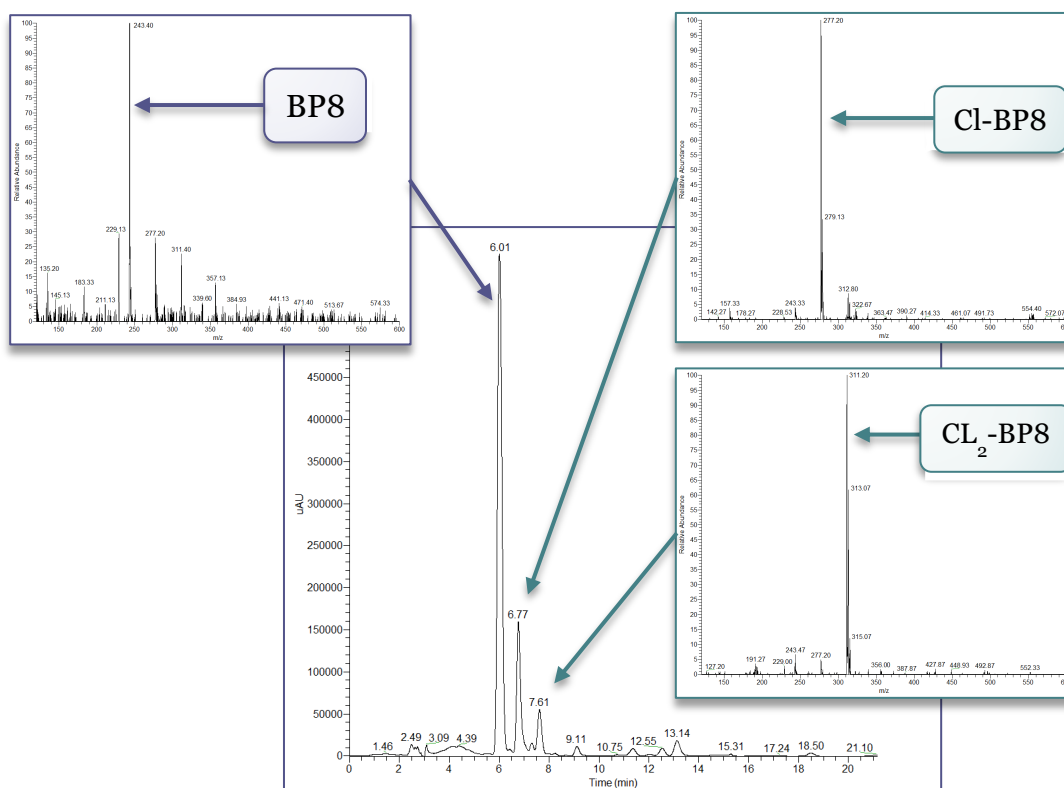


Figure 4. Total-ion current (TIC) chromatogram for the degradation reaction of BP8 with free active chlorine, and the compounds successfully determined in the process.

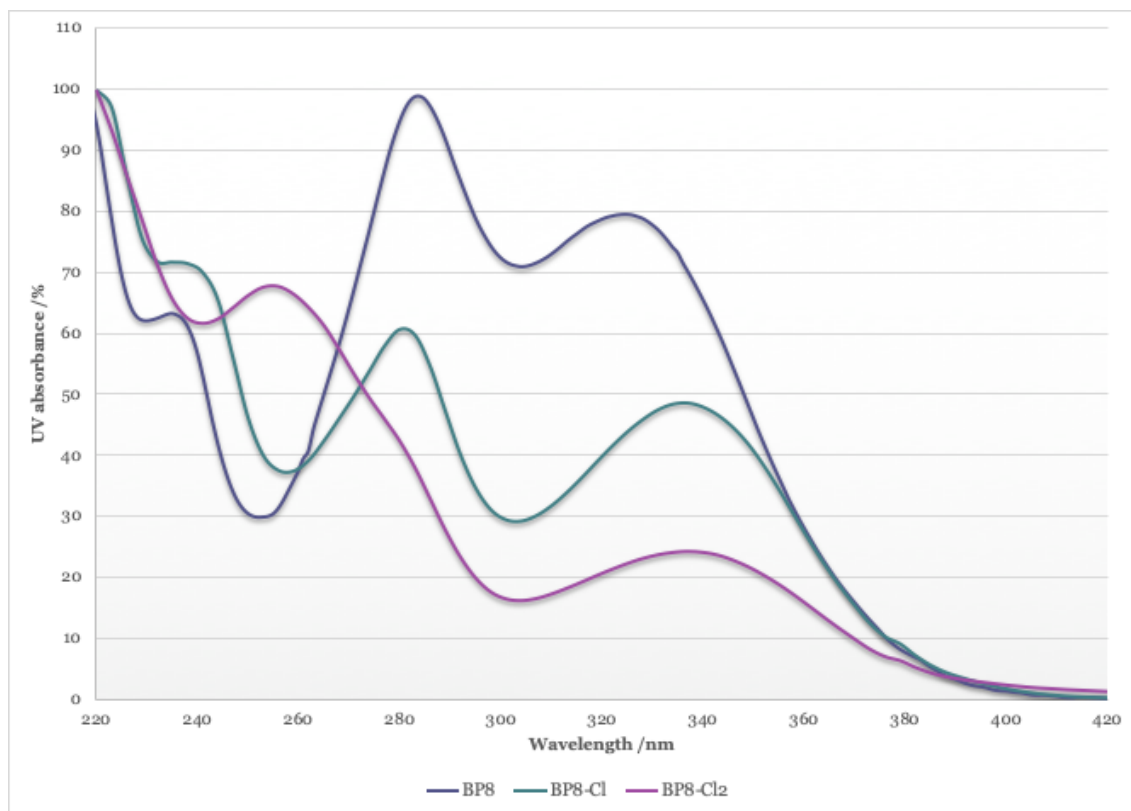


Figure 5. UV spectra of the parental compound BP8 and its DBPs, the monochlorinated and dichlorinated forms of the molecule.

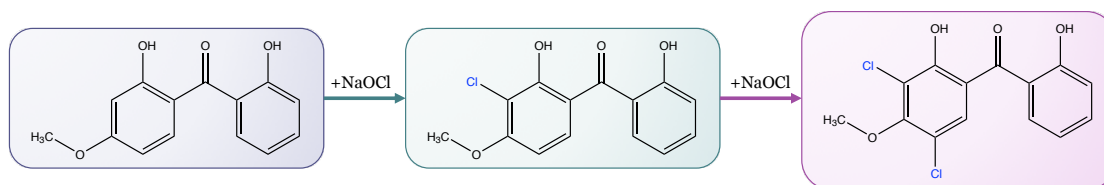


Figure 6. Most probable paths for the electrophilic substitution of chlorine on the molecule, specifically around the strongly *ortho* and *para* directive -OH and -OCH_3 functional groups.

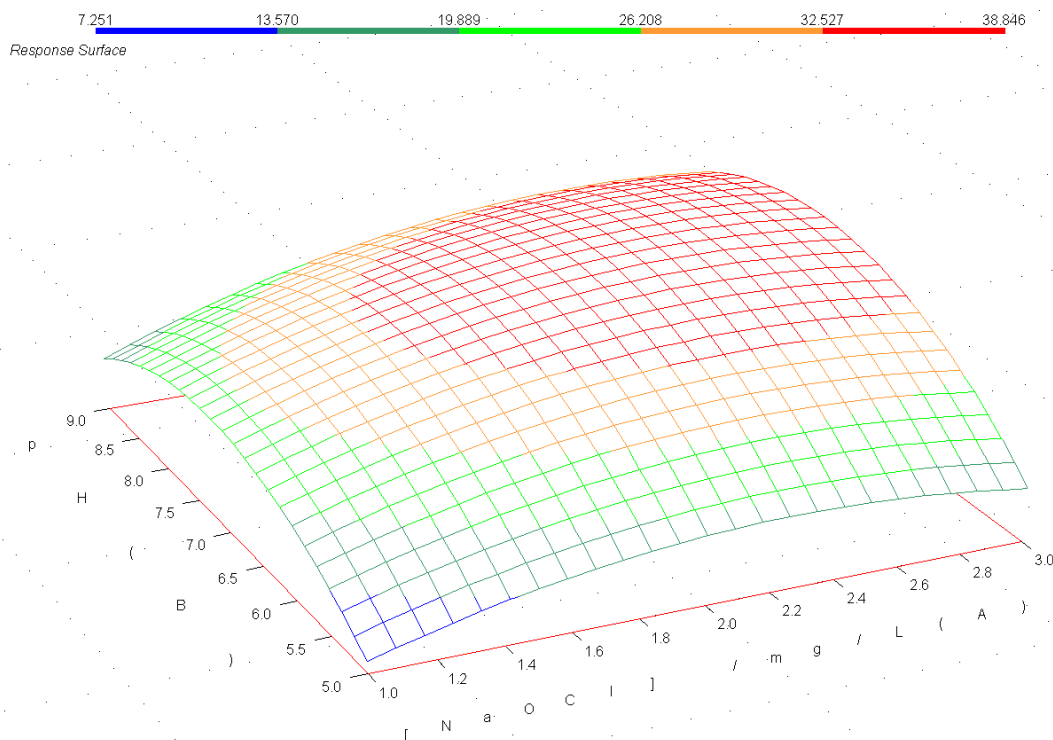


Figure 7. Response surface for Set A of experiments (without DOM in solution), determined as a function of the interaction between NaOCl concentration and the pH values.

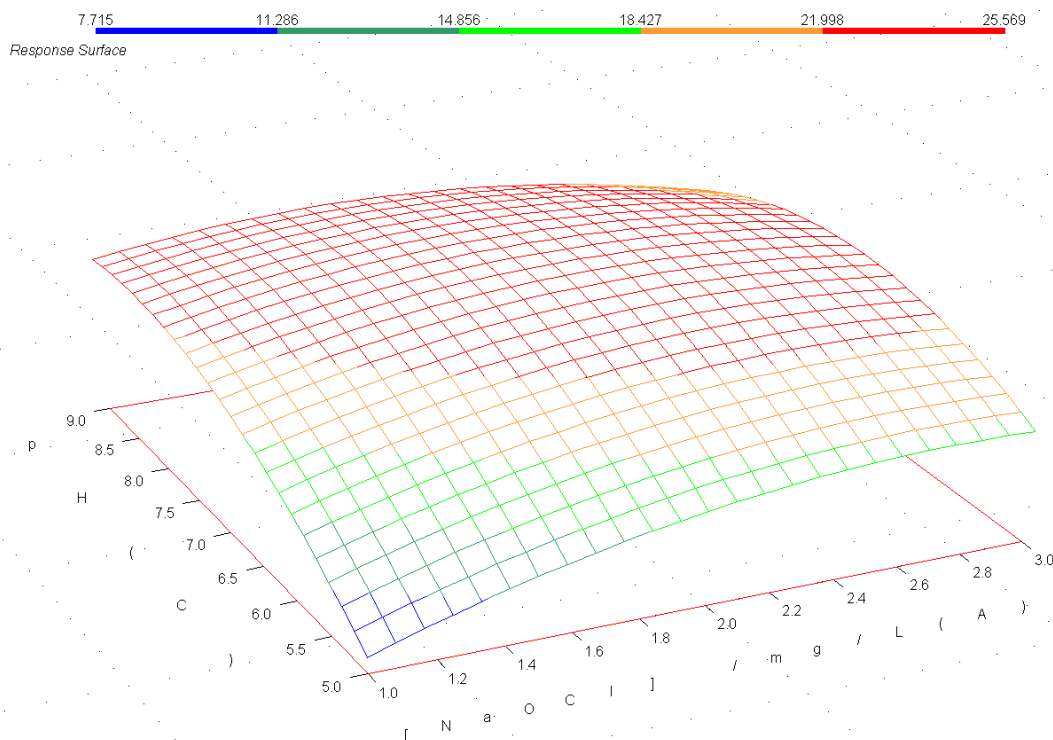


Figure 8. Response surface for Set B of experiments (with DOM in solution), obtained as a function of the interaction between NaOCl and pH.

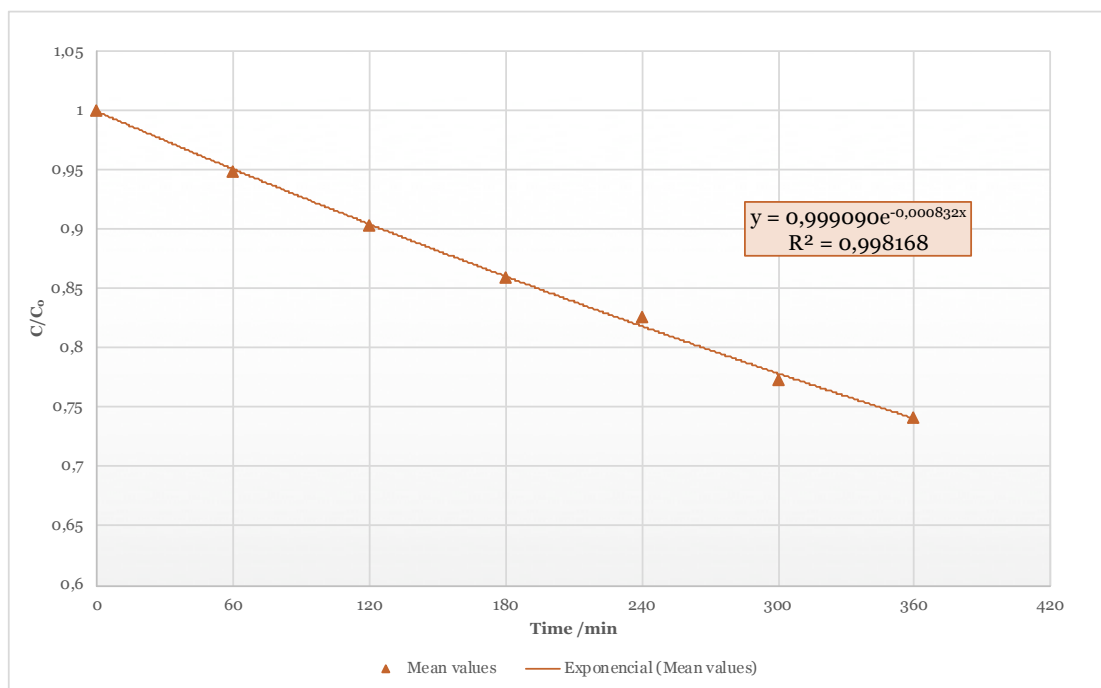


Figure 9. Exponential plot of the relative concentration of BP8 as a function of artificial irradiation time.

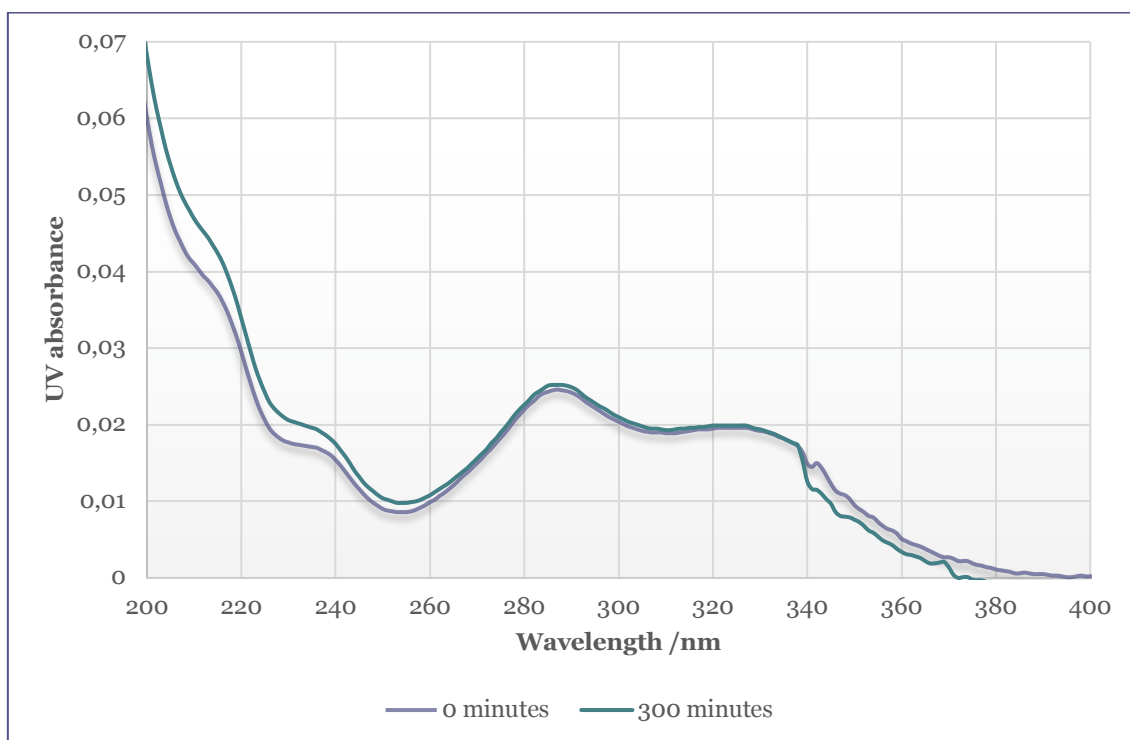


Figure 10. UV spectra alterations undergone by BP8 throughout artificial irradiation.

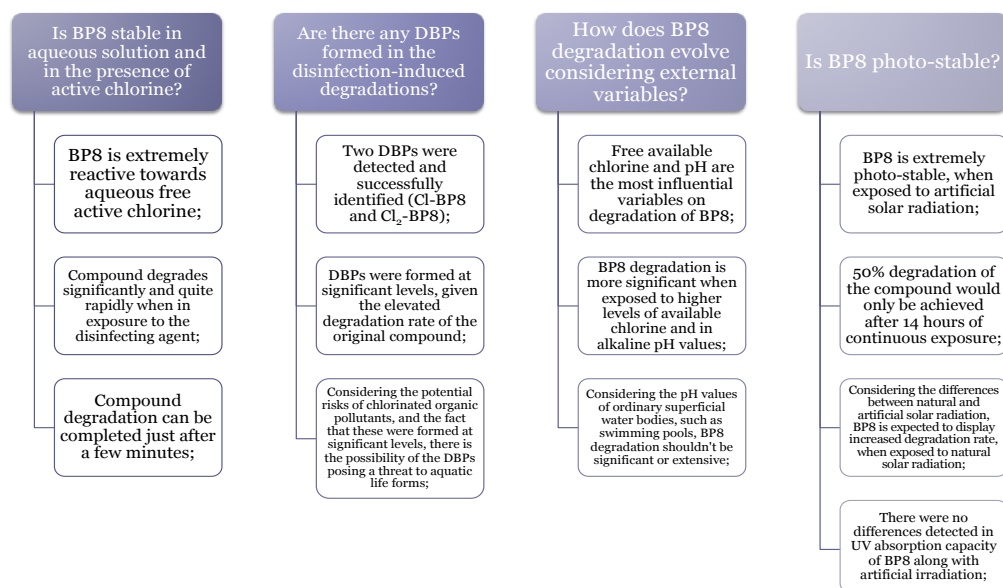


Figure 11. Final conclusions about the fate and behavior of BP8 in the aquatic environment.

Supplementary material

Environmental fate and behaviour of benzophenone-8 in aqueous solution

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1. Identification of DBPs

The molecular structures and fragmentation paths for the most important and abundant molecular ions and fragments, are presented in [Figures S1, S2 and S3](#), in the case of BP8, its monochlorinated and dichlorinated DBPs, respectively. The mass spectra for each compound and fragmentations are included in [Figures S4 to S9](#). Identification details are as follows (the peaks referred in the following details, are those identified in the chromatogram in [Figure 4](#) of the main manuscript):

- Peak at 6.01 minutes (identified as the parental compound, BP8): the mass spectrum yielded the m/z value of 243.40 (100%), consistent with the molecular mass of the compound (244.2427 g/mol) in MS negative mode;
 - The fragmentation of this peak originated the molecular ions 199.20 (100%); 123.27 (25%); 93.27 (23%); 184.27 (12%); 224.87 (10%); and 211.27 (8%);
- Peak at 6.77 minutes (identified as the monochlorinated form of BP8, Cl-BP8): the mass spectrum of this peak showed an m/z value of 277.20 (100%), consistent with the molecular mass of the monochlorinated form BP8 (278.6878 g/mol) in MS negative mode;
 - Fragmentation of this peak yielded the molecular ions 157.20 (100%); 233.07 (85%); 218.07 (20%); 234.20 (16%); and 258.93 (10%);
- Peak at 7.61 minutes (identified as the dichlorinated form of BP8, Cl₂-BP8): the mass spectrum of this peak yielded the m/z value of 311.20 (100%), consistent with the molecular mass of the dichlorinated form of BP8 (313.5770 g/mol) in MS negative mode;
 - Fragmentation of this peak led to the molecular ion 191.00 (100%);

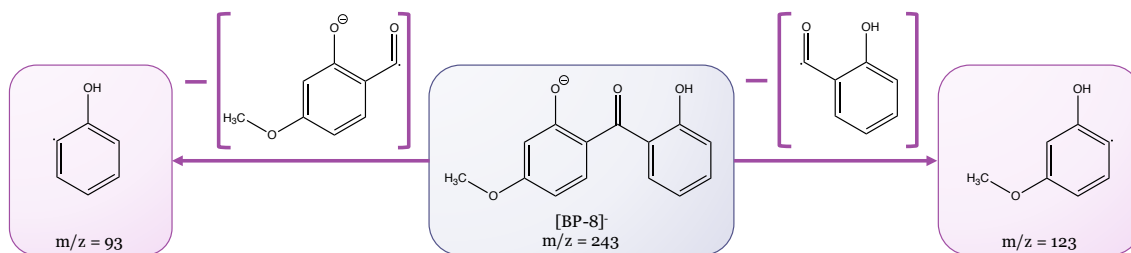


Figure S1. Proposed fragmentation paths for the most prominent molecular ions of the parental compound, BP8.

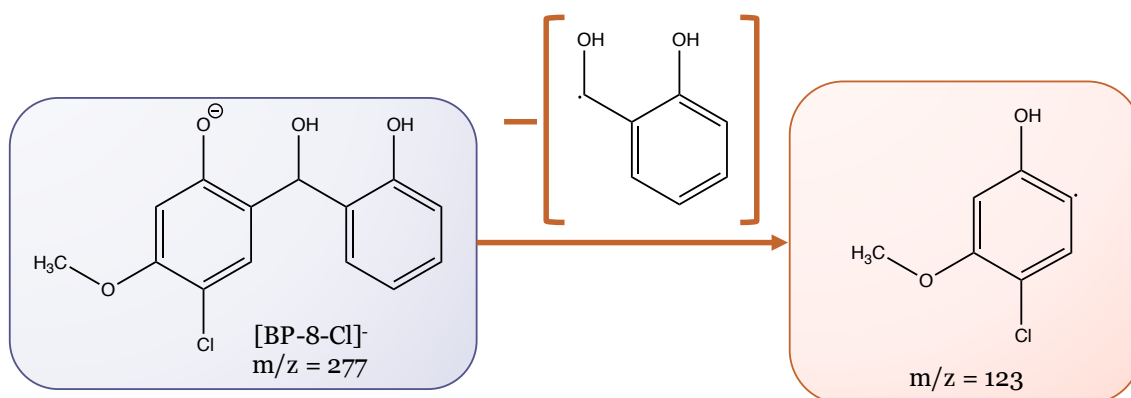


Figure S2. Proposed fragmentation paths for the most prominent molecular ions of the monochlorinated form of BP8 (Cl-BP8).

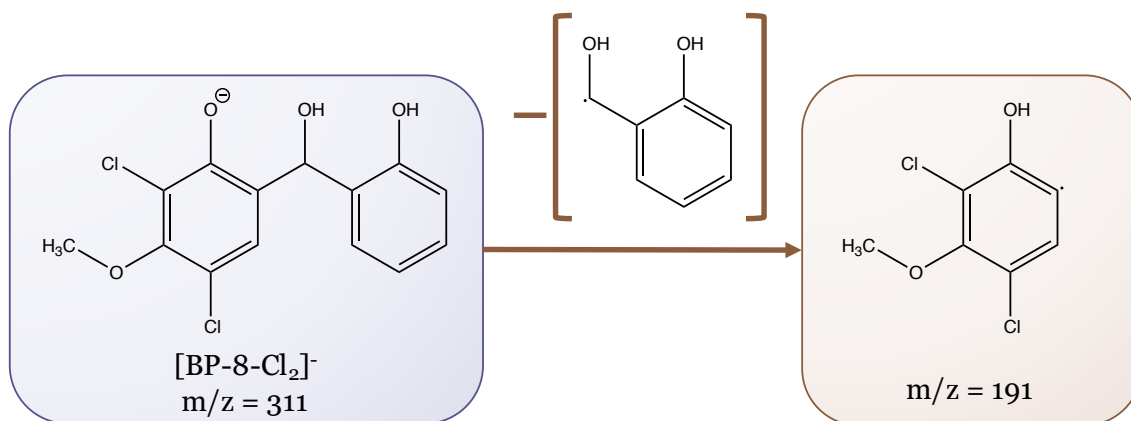


Figure S3. Proposed fragmentation paths for the molecular ion of the dichlorinated form of BP-8 (Cl_2 -BP8).

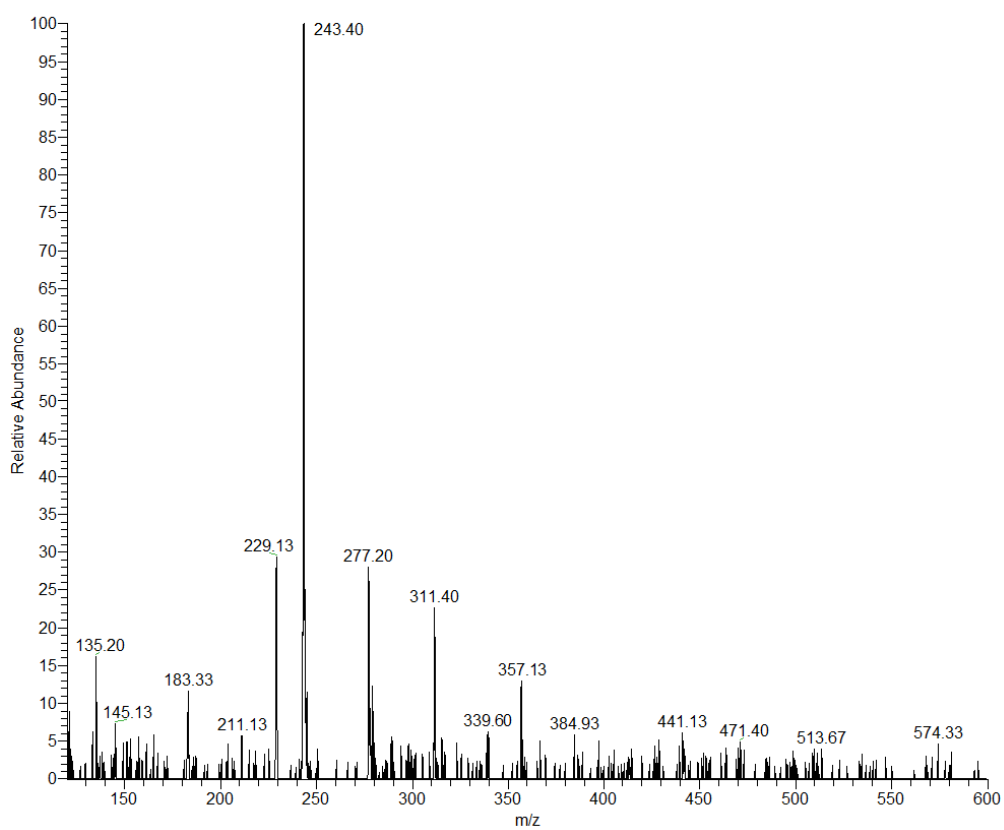


Figure S4. Mass spectrum obtained for the parental compound, BP8.

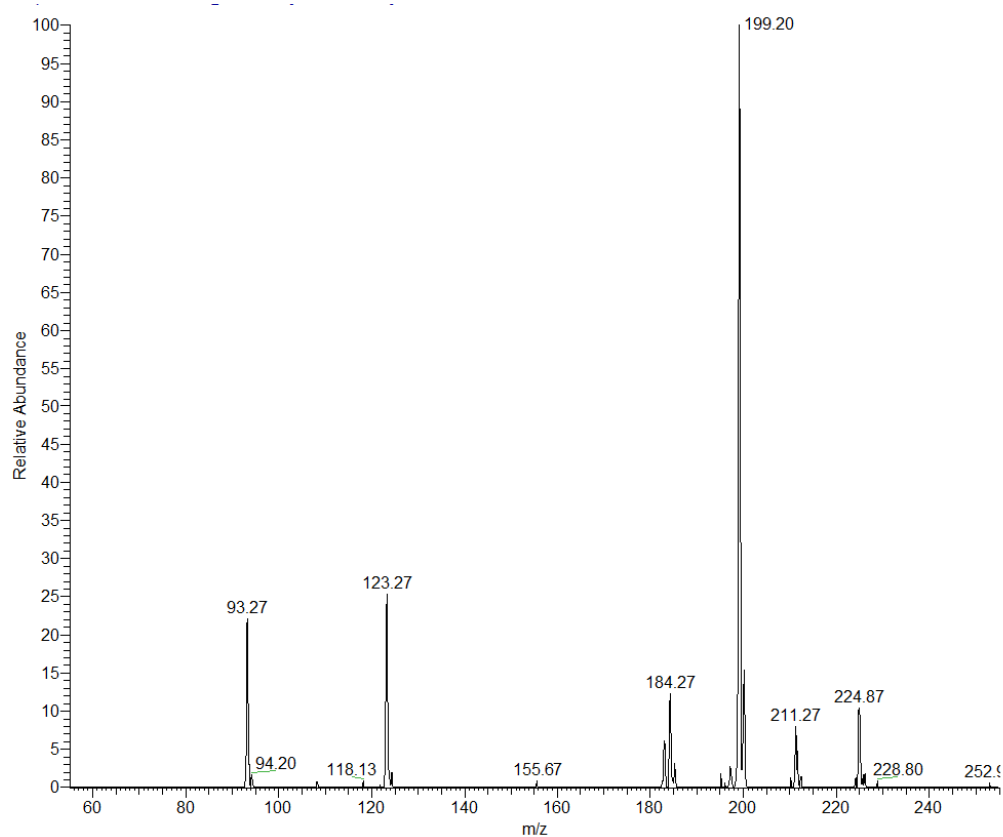


Figure S5. Fragmentation of the parental compound, BP8, and all the molecular ions obtained.

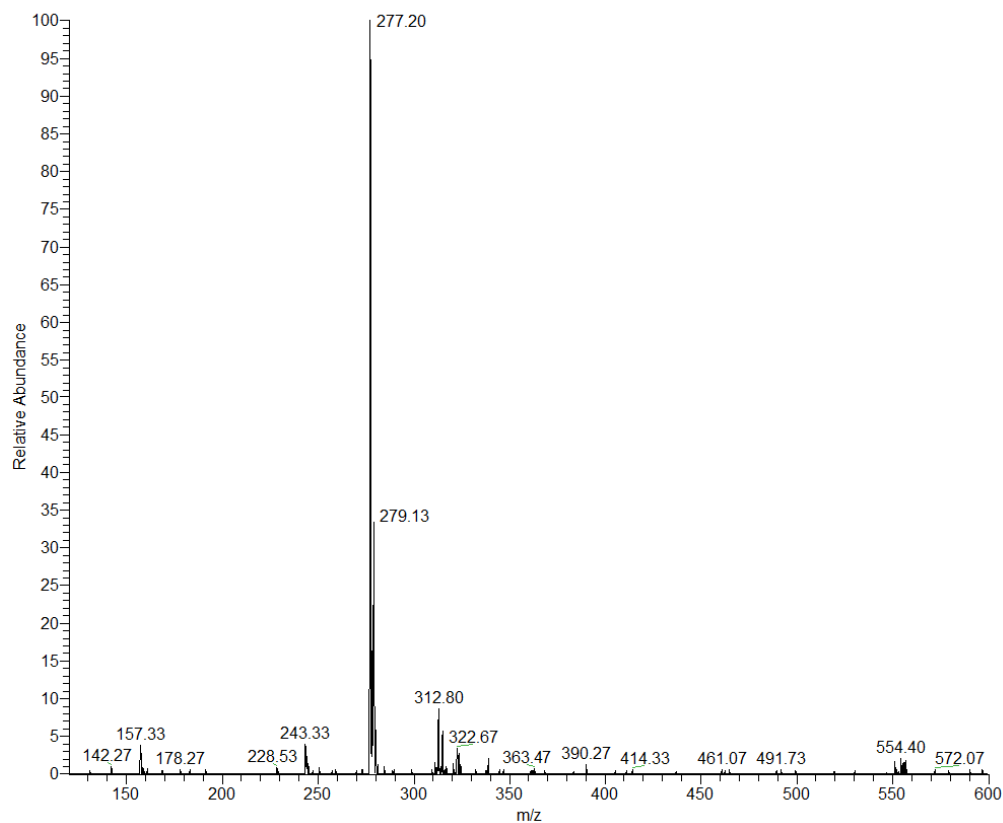


Figure S6. Mass spectrum obtained for the monochlorinated form of BP8, Cl-BP8.

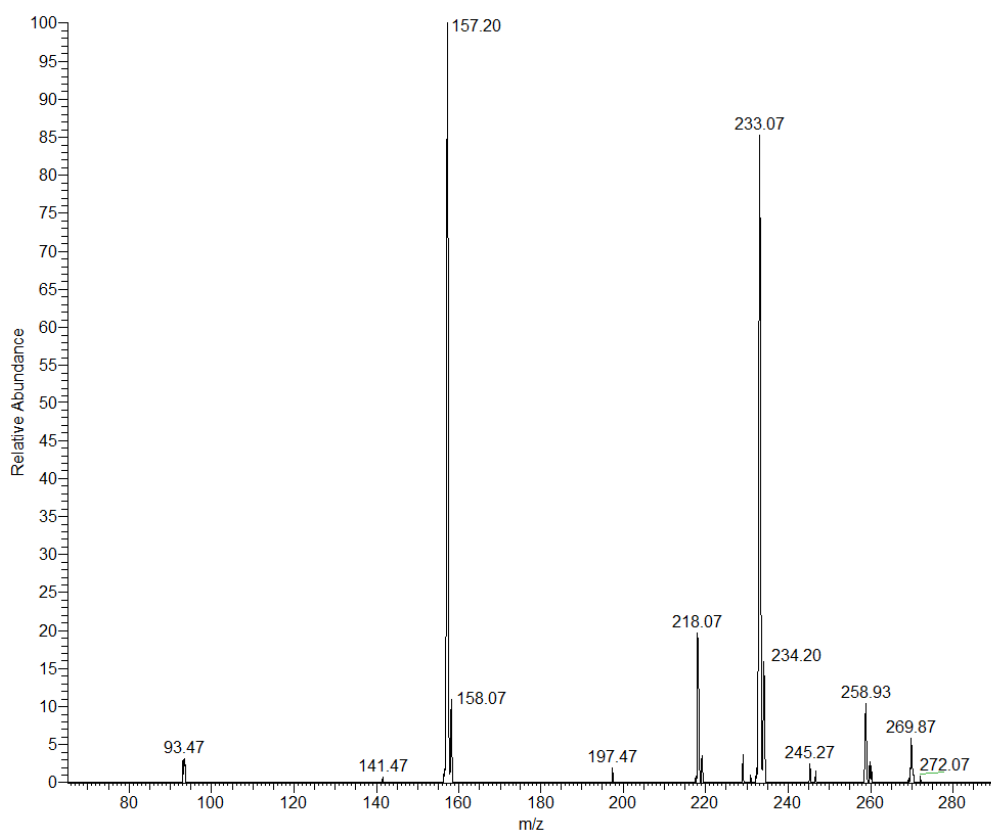


Figure S7. Fragmentation of the monochlorinated form of BP8, Cl-BP8, and all the molecular ions obtained.

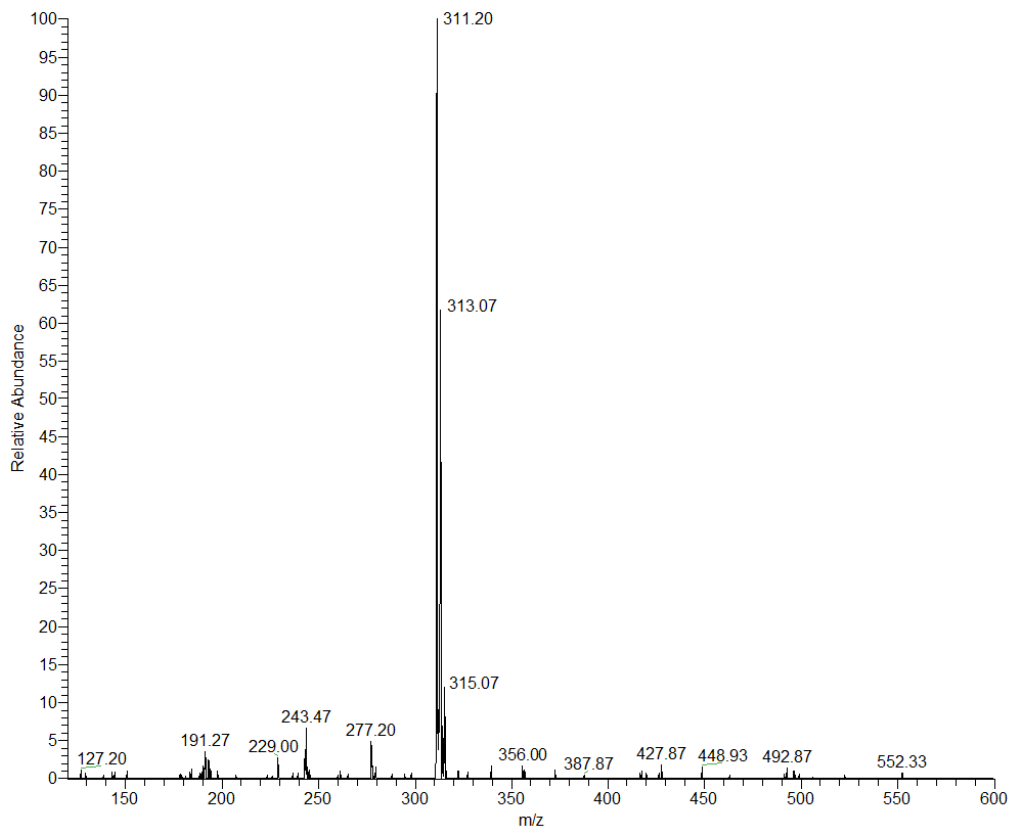


Figure S8. Mas spectrum obtained for the dichlorinated form of BP8, Cl₂-BP8.

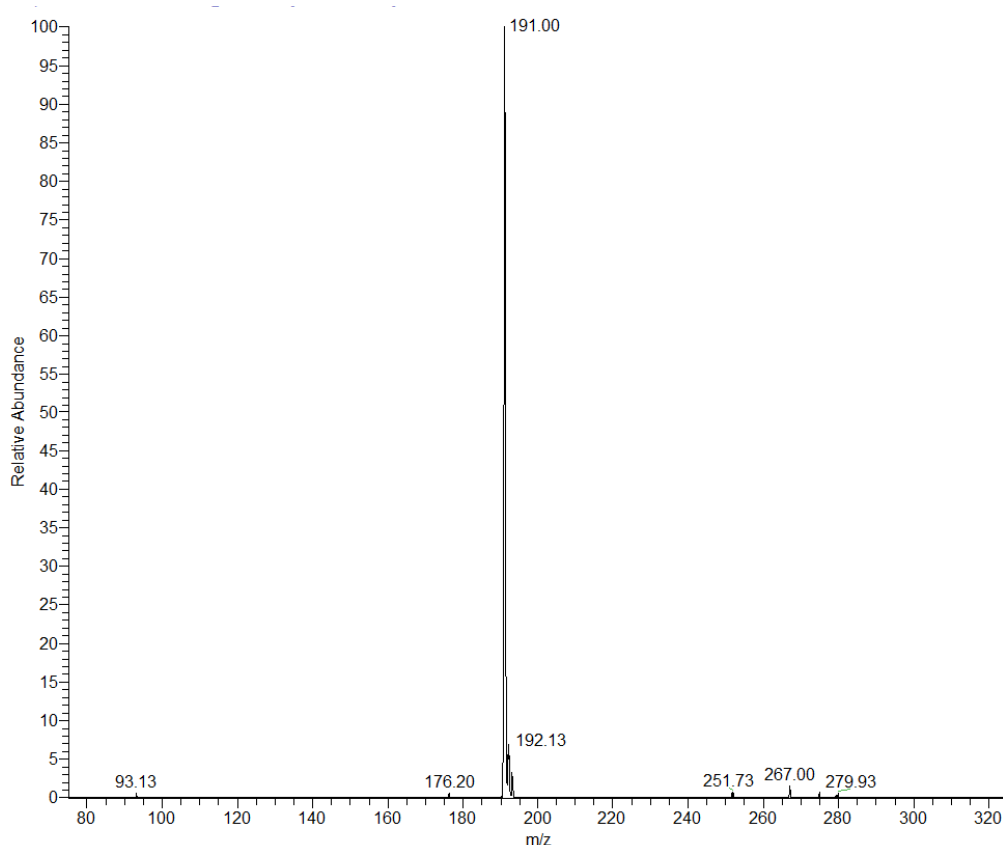


Figure S9. Fragmentation of the dichlorinated form of BP8, Cl₂-BP8, and all the molecular ions obtained.

2. Influence of external variables on degradation

The response surfaces for sets A and B, are presented in [Figures S11](#) and [S12](#), respectively. Detailed information about both sets of experiments are presented in section 2.3.3 and [Figure 2](#), on the main manuscript. Complete overview of experimental conditions, are included in [Tables S1](#) and [S2](#).

Table S1. Experimental conditions for the first Box-Behnken design (Set A of experiments).

Experiência	[BP8] /mg/L	[NaOCl] /mg/L	pH	T /°C
1	0,5002	1	5	20
1b	0,5002	1	5	20
2	0,5002	3	5	20
2b	0,5002	3	5	20
3	0,5002	1	9	20
3b	0,5002	1	9	20
4	0,5002	3	9	20
4b	0,5002	3	9	20
5	0,5002	1	7	15
5b	0,5002	1	7	15
6	0,5002	3	7	15
6b	0,5002	3	7	15
7	0,5002	1	7	25
7b	0,5002	1	7	25
8	0,5002	3	7	25
8b	0,5002	3	7	25
9	0,5002	2	5	15
9b	0,5002	2	5	15
10	0,5002	2	9	15
10b	0,5002	2	9	15
11	0,5002	2	5	25
11b	0,5002	2	5	25
12	0,5002	2	9	25
12b	0,5002	2	9	25
Central a	0,5002	2	7	20
Central b	0,5002	2	7	20

Table S2. Experimental conditions for the second Box-Behnken design (Set B of experiments).

Experiência	[BP-8] /mg/L	[NaOCl] /mg/L	[DOM] /mg/L	pH	T /°C
1	0,5002	1	1,0000	7	20
1b	0,5002	1	1,0000	7	20
2	0,5002	3	1,0000	7	20
2b	0,5002	3	1,0000	7	20
3	0,5002	1	5,0000	7	20
3b	0,5002	1	5,0000	7	20
4	0,5002	3	5,0000	7	20
4b	0,5002	3	5,0000	7	20
5	0,5002	1	2,5500	5	20
5b	0,5002	1	2,5500	5	20
6	0,5002	3	2,5500	5	20

6b	0,5002	3	2,5500	5	20
7	0,5002	1	2,5500	9	20
7b	0,5002	1	2,5500	9	20
8	0,5002	3	2,5500	9	20
8b	0,5002	3	2,5500	9	20
9	0,5002	1	2,5500	7	15
9b	0,5002	1	2,5500	7	15
10	0,5002	3	2,5500	7	15
10b	0,5002	3	2,5500	7	15
11	0,5002	1	2,5500	7	25
11b	0,5002	1	2,5500	7	25
12	0,5002	3	2,5500	7	25
12b	0,5002	3	2,5500	7	25
13	0,5002	2	1,0000	5	20
13b	0,5002	2	1,0000	5	20
14	0,5002	2	5,0000	5	20
14b	0,5002	2	5,0000	5	20
15	0,5002	2	1,0000	9	20
15b	0,5002	2	1,0000	9	20
16	0,5002	2	5,0000	9	20
16b	0,5002	2	5,0000	9	20
17	0,5002	2	1,0000	7	15
17b	0,5002	2	1,0000	7	15
18	0,5002	2	5,0000	7	15
18b	0,5002	2	5,0000	7	15
19	0,5002	2	1,0000	7	25
19b	0,5002	2	1,0000	7	25
20	0,5002	2	5,0000	7	25
20b	0,5002	2	5,0000	7	25
21	0,5002	2	2,5500	5	15
21b	0,5002	2	2,5500	5	15
22	0,5002	2	2,5500	9	15
22b	0,5002	2	2,5500	9	15
23	0,5002	2	2,5500	5	25
23b	0,5002	2	2,5500	5	25
24	0,5002	2	2,5500	9	25
24b	0,5002	2	2,5500	9	25
Central a	0,5002	2	2,5500	7	20
Central b	0,5002	2	2,5500	7	20
Central c	0,5002	2	2,5500	7	20

The analysis of variance (ANOVA) information of Set A (without DOM in solution), is presented in Table S3. The variables studied were NaOCl concentration, the pH, water temperature and all their quadratic terms and interactions. The statistical validation of the model is as follows:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the critical F -value (obtainable from the distribution tables of statistical literature) is 1.833;
- As presented in Table S3, the experimental F -value of the model is 203.900, and therefore, overwhelmingly higher than the critical F -value just mentioned (1.833);
- Additionally, the P -value for the model is 0, and therefore lower than the significance level ($\alpha = 0.05$);

The experimental P -value (0.0060) regards the error of the model, and it is lower than the chosen significance level (0.05), which means that the obtained results are therefore proven to display statistical significance. Regarding the experimental F -value (203.900), which compares the combined effect of the variables, it is quite higher than the critical or theoretical value for that level of significance (1.833). In other words, the relation between the variables and the obtained results is indeed also statistically significant. In conclusion, the data allows for the rejection of the null hypothesis (H_0), which states that there is no interaction or relation between the studied variables and that the differences found within the results are not statistically relevant, and it allows, therefore, the acceptance of the alternative hypothesis (H_a), that defends the exact opposite, that is, the results and the interaction between the variables are statistically significant. As for the results, the most influential variables proved to be the NaOCl concentration (β -coefficient = 6.018 ± 0.298) and the pH (β -coefficient = 2.958 ± 0.149). In parallel, these are also the factors with the highest F -values (406.965 for the NaOCl concentration, and 393.220 for the pH). The experimental P -values for both these terms also fall below the significance level of 0.05.

Table S3. ANOVA data for Set A (without DOM in solution) on the study of influence of external variables on the degradation of BP8.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	2613	9	290.337	203.900	0		
	Error	24.207	17	1.424				
	Adjusted total	2637	26	101.432				
Factors	Intercept	4013	1	4013	2818	0	36.573	0.689
	[NaOCl] /mg/L (A)	579.485	1	579.485	406.965	0	6.018	0.298
	pH (B)	559.914	1	559.914	393.220	0	2.958	0.149
	T /°C (C)	0.03063	1	0.03063	0.02151	0.8851	-0.008750	0.05966
	AB	38.852	1	38.852	27.285	0.0001	1.356	0.260
	AC	363.151	1	363.151	255.036	0	4.146	0.260
	BC	29.722	1	29.722	20.873	0.0003	-1.186	0.260
	AA	257.374	1	257.374	180.750	0	-4.154	0.309
	BB	931.157	1	931.157	653.939	0	-7.902	0.309
	CC	307.963	1	307.963	216.278	0	-4.544	0.309
Model check	Main	1139	3	379.810				
	Int	431.725	3	143.908	101.065	0		
	Int + Sqr	1042	3	347.294	243.900	0		
	Squ	1042	3	347.294	243.900	0		
	Error	24.207	17	1.424				
Lack of fit	Lack of fit	23.557	3	7.852	169.334	0		
	Pure error	0.649	14	0.04637				
	Total error	24.207	17	1.424				

As far as Set B is concerned (with DOM included in solution), the ANOVA data is presented in Table S4. The variables studied were the same as in Set A with the addition of DOM concentration. The statistical validation of the model was as follows:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the critical F -value (obtainable from the distribution tables of statistical literature) is 1.761;
- As presented below in Table S4, the experimental F -value of the model is 15.632, and therefore, higher than the critical F -value just mentioned above (1.761);
- The P -value for the model is 0, and therefore lower than the significance level (0.05);

As was explained above, the experimental P -value (0) is lower than the chosen significance level of the model (0.05), and subsequently, the results display statistical significance. Additionally, the experimental F -value (15.632), is higher than the critical value (1.761), which means that the interaction between the variables and the relation between the obtained results, are also statistically significant. Once again, data allows for the safe rejection of the null hypothesis (H_0) in favour of the alternative hypothesis (H_a). The most influential variables on the degradation of BP-8 in Set B, and just like in the case of Set A, were the NaOCl concentration (β -coefficient = 1.684 ± 0.450) and the pH (β -coefficient = 1.953 ± 0.225). As in the case of the first model, these variables were also the ones with the highest F -values (13.992 for NaOCl concentration and 75.226 for the pH). Their experimental P -values (0.0006 for the NaOCl concentration and 0 for the pH) were also found to be lower than the significance level of 5%.

Table S4. ANOVA data for the Set B (with DOM in solution) on the study of influence of external variables on the degradation of BP-8.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1065	14	76.050	15.632	0		
	Error	175.142	36	4.865				
	Adjusted total	1240	50	24.797				
Factors	Intercept	1846	1	1846	379.362	0	24.803	1.273
	[NaOCl] /mg/L (A)	68.074	1	68.074	13.992	0.0006	1.684	0.450
	[DOM] /mg/L (B)	7.150	1	7.150	1.470	0.2333	-0.273	0.225
	pH (C)	365.977	1	365.977	75.226	0	1.953	0.225
	T /°C (D)	4.699	1	4.699	0.966	0.3323	-0.08850	0.09005
	AB	5.136	1	5.136	1.056	0.3111	-0.385	0.374
	AC	84.175	1	84.175	17.302	0.0002	-1.557	0.374
	AD	36.040	1	36.040	7.408	0.0099	1.019	0.374
	BC	2.112	1	2.112	0.434	0.5142	-0.247	0.374
	BD	27.677	1	27.677	5.689	0.0225	0.893	0.374
	CD	2.333	1	2.333	0.480	0.4931	0.259	0.374
	AA	74.451	1	74.451	15.303	0.0004	-1.524	0.390
	BB	260.574	1	260.574	53.560	0	-2.851	0.390
	CC	190.586	1	190.586	39.175	0	-2.439	0.390
	DD	384.038	1	384.038	78.938	0	-3.462	0.390
Model check	Main	445.900	4	111.475				
	Int	157.472	6	26.245	5.395	0.0005		
	Int + Sqr	461.334	4	115.333	23.707	0		
	Squ	461.334	4	115.333	23.707	0		
	Error	175.142	36	4.865				
Lack of fit	Lack of fit	174.412	10	17.441	621.093	0		
	Pure error	0.730	26	0.02808				
	Total error	175.142	36	4.865				

Chapter 3 - Degradation studies of selected pharmaceuticals

Fate and behaviour of prominent pharmaceuticals, paracetamol, 17 α -ethynylestradiol and carbamazepine, in aqueous solution

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Abstract

Pharmaceuticals are invaluable tools for the prevention and treatment of human and animal diseases. Social, technological and economic development promotes the increase in life expectancy, and subsequently, the increase of the population on the planet. This also induces the significant increase in the requirement and application of human and veterinarian pharmaceuticals. Interest on the occurrence, fate and behaviour of pharmaceuticals in superficial waters, has also increased significantly, due to the continuous detection of these compounds in the aquatic, environmental compartments. This work focuses on the thorough investigation of the degradation scenarios typically undergone by these compounds, namely the disinfection-induced and photo-induced degradation reactions, which not only mitigate or eliminate the pollutants from the water bodies but are also believed to yield the formation of potentially hazardous degradation products. In superficial waters used for human or animal purposes, water disinfection is commonly carried out in municipal water treatment infrastructures, typically by using chlorine-based disinfectants, also known to promote the disinfection-induced reactions and therefore, the formation of the so-called disinfection by-products (DBPs). These compounds are chlorinated organic structures, with concerning eco-toxicological potential. Photo-induced degradation typically destroys or alters the structure of the parental compounds, yielding the formation of photo-isomers or highly reactive particles. Three very popular pharmaceuticals, namely acetaminophen (an important anti-inflammatory also known as paracetamol), 17 β -ethinylestradiol (a popular synthetic estrogen used in birth-control pills) and carbamazepine (an anti-epileptic), were meticulously studied in aqueous solution, in order to determine their degradation rates and half-life periods, as well as identifying potential transformation by-products. The investigation of the effect of external variables on the degradation reactions, was also assessed, to determine how they proceed in case of variable pH, water temperature, chlorine concentrations and the presence or absence of naturally-dissolved organic matter (DOM).

Keywords

Emerging pollutants; EPs; pharmaceuticals; paracetamol; acetaminophen; carbamazepine; CBZ; 17 α -ethinylestradiol; EE2; disinfection by-products; DBPs;

1. Introduction

Pharmaceuticals represent one of the most important sub-classes of the so-called emerging pollutants (EPs), whose very name hints at the character of the compounds in case and their increasing environmental prevalence. In fact, EPs are being recurrently detected in superficial waters worldwide [1, 2], consequence of the exponential increase in their use and application in numerous contexts and also their highly complex routes of inclusion into the environment [3].

By their very definition, EPs have been seldom studied as to their fate and behaviour in aqueous solution, they are believed to represent nefarious risk towards the health of the general environmental compartments [4, 5], especially the superficial waters and its corresponding non-targeted life-forms, and last but not least, they are yet unbound by water-safety regulation or legislation [3, 6]. On the other hand, subsequently to overwhelming social, economic and technological development, current life expectancy is unprecedentedly high and populations keep rising, whilst health-care investment and coverage is increasingly prominent, which means that access to and consumption of pharmaceuticals has also grown exponentially [1].

With consumption growth, environmental levels of pharmaceuticals are also expected to rise significantly. In some cases, these have actually exceeded the predicted no-effect concentrations (PNEC), as was proven in extreme for the popular anti-inflammatory diclofenac, which was found above the PNEC levels in more than 30 countries [2], but also with other compounds, like antibiotics such as trimethoprim and ciprofloxacin or anti-inflammatories such as ibuprofen [7]. Additionally, pharmaceuticals are known to display considerable proclivity towards environmental persistence and bioaccumulation [4], which further highlights the nefarious risks of exposure of non-target organisms and the fragile ecosystems towards environmentally-relevant concentrations of these compounds [1].

There are many natural processes for mitigation or elimination of these pollutants from natural, superficial or ground water bodies, typically phenomena of chemical, biological and photo-chemical degradation, which explains why levels are expected to be higher at waste water treatment facilities [7]. However, the processes of natural mitigation are strongly dependant on the compounds' physical-chemical properties, which, in many cases, renders them highly resistant towards attenuation [7]. In

municipal waters, elimination is provided in water treatment facilities, although the process is also inefficient in quantitatively eliminating the pollutants from the water courses [2].

As far as the risks are concerned, several studies conducted in the laboratory, in the field and even in ecosystem-scaled contexts, have linked exposure to pharmaceuticals, directly towards serious eco-toxicological implications [1, 7]. Exposure to diclofenac affected subjects of rainbow trout fish, causing serious renal and intestinal afflictions, as well as alterations on the gills and hepatic gene expression [8-10]. Ibuprofen, carbamazepine and sulfamethoxazole, all demonstrated toxicity towards algae, with effects in chloroplasts being detected after exposure to ibuprofen and carbamazepine [11], and chronic toxicity for the photo-synthetic apparatus, being detected after exposure to sulfamethoxazole [12]. The synthetic estrogen used in oral contraceptives, 17 α -ethynylestradiol, was also shown to cause serious effects in steroidogenic organs of Atlantic salmon subjects [13]. In even more extreme examples, diclofenac was proved to be the compound responsible for the near extinction of an entire vulture population in Pakistan, after the pharmaceutical was administered to cows, in order to treat symptoms of fever, and the vultures started feeding on their corpses later on [14]. The collapse of an entire population of fathead minnows, was also nearly achieved in a study at the Experimental Lakes Area, in Ontario, when the subjects were exposed to 17 α -ethynylestradiol and it eventually caused serious feminization of an overwhelming rate of male subjects [15].

All the aforementioned studies, highlight the need to study the fate and behaviour of pharmaceuticals in the aquatic context, as well as continuing to investigate their potential implications towards non-target organisms and the environment in general. The present work will address the reactivity of three prominent pharmaceuticals (the anti-inflammatory acetaminophen, also known as paracetamol; the synthetic estrogen 17 α -ethynylestradiol, used in oral contraceptives; and the anti-epileptic carbamazepine) in aqueous solution. The molecular structures of the three compounds are presented on Figure 1, and their relevant physical-chemical properties are included in Table 1. The aim is to determine the kinetic features of the compounds, in particular their half-life periods in the context of disinfection-induced and photo-induced degradation. Particular attention will also be drawn to the identification of potential by-products of the degradation reactions. Additionally, the influence of external, relevant

environmental variables, such as pH, temperature, active chlorine concentration and the presence/absence of dissolved organic matter (DOM), will be ascertained by means of a Box-Behnken design formulation with multi-factorial analysis and response surface methodology. Degradation reactions will be followed by a robust and reliable high-performance liquid chromatography system coupled with ultraviolet (UV) diode-array detection (HPLC-UV-DAD). Photo-induced degradation will be ensured by means of artificial solar radiation with the help of a solar radiation simulator with air mass filters and a liquid light guide. Determination of potential by-products will be carried out by use of a highly sensitive and accurate liquid chromatography system, coupled with diode-array detection and mass spectrometry (LC-DAD-MS). To additionally enhance and improve sensitivity and accuracy of the method, nonetheless, a protocol of solid-phase extraction (SPE) was always carried out before the LC-DAD-MS studies and analysis.

2. Materials and methods

2.1. Reagents

The pharmaceuticals N-(4-hydroxyphenyl)-acetamide (also known as acetaminophen or paracetamol; CAS number 103-90-2), 17 α -ethynyl-1,3,5-(10)-estratriene-3,17 β -diol (also known as 17 α -ethynylestradiol; CAS number 57-63-6) and 5H-Dibenz[b,f]azepine-5-carboxamide (also known as carbamazepine; CAS number 298-46-4) were all purchased from Sigma-Aldrich, Merck, Darmstadt, Germany. A reagent-grade sodium hypochloride (NaOCl) solution, with 3.5% active chlorine labelled content, was purchased from VWR Chemicals, Radnor, PA, USA. From this reagent solution, a stock solution of 432 mg/L in active chlorine content was prepared and permanently stored at 4°C and removed only prior to use. Active chlorine content was evaluated by means of the standard iodometric titration method. The compound L-ascorbic acid at 99%, used to prepare the ascorbic acid standard solutions, was purchased from Merck, Darsmtadt, Germany. The only water source used for every purpose in every experiment, was deionised water (conductivity < 0.1 μ S/cm). If or when required, pH regulation or adjustment was always ensured by the addition of ordinary standard solutions of either hydrochloric acid (HCl) or sodium hydroxide

(NaOH). Pre-concentration of the aqueous reaction mixtures, necessary for the analysis and identification of potential by-products by liquid chromatography coupled with diode-array detection and mass spectrometry (LC-DAD-MS), was carried out by solid-phase extraction (SPE) with LiChrolut EN 200 mg, 6 mL standard cartridges from Merck, Darmstadt, Germany. All organic solvents used for pre-concentration or chromatographic purposes, namely methanol, acetonitrile or dichloromethane, were of liquid chromatographic-grade and purchased from Merck, Darmstadt, Germany. Dissolved organic matter (DOM) was obtained from pinewood soil [16-18].

2.2. Chromatographic conditions

2.2.1. HPLC-UV-DAD

High-performance liquid chromatography coupled with ultraviolet diode array detection (HPLC-UV-DAD), was used to follow all the degradation reactions along with time. This system was assembled with an isocratic pump (Hewlett–Packard 1100 Series, Böblingen, Germany), a manual sample injection valve with a 20 μ L loop (Rheodyne 7725i, Rohnert Park, USA), the corresponding silica-based, reverse-phase column (a Dionex Acclaim™ 120 C18, particle size 3 μ m and pore diameter 175 Å [4.6×150 mm], from Thermo-Fisher Scientific, Sunnyvale, CA, USA, for carbamazepine [CBZ] and 17 α -ethynylestradiol [EE2] analysis, or a Supelcosil LC-18, particle size 5 μ m, 4.6 x 250 mm, from Supelco, Sigma-Aldrich, Merck, Darmstadt, Germany, for paracetamol [PAR] analysis), and a photo-diode array detector (UV 6000LP with a 50 mm LighPipe flow cell, from Thermo-Fisher Scientific, Sunnyvale, CA, USA). The mobile phases used to study and follow the degradation reactions, were as follows:

- For paracetamol (PAR) analysis, a deionised water, formic acid (HCOOH) and liquid chromatography-grade methanol mixture (79:1:20, v:v), at a 0.50 mL/min flow-rate;
- For 17 α -ethynylestradiol (EE2) analysis, a liquid chromatography-grade acetonitrile and deionised water mixture (60:40, v:v), at a 0.50 mL/min flow-rate;
- For carbamazepine (CBZ) analysis, a liquid chromatography-grade acetonitrile and deionised water mixture (60:40, v:v) at a 0.40 mL/min flow-rate;

Before application, all mobile phases were always filtered by pressure through a borosilicate glass funnel (pore size 4-8 microns). Ultraviolet (UV) absorbances were monitored and recorded at a total scan mode (200-600 nm), and the entire system was controlled with the Xcalibur 1.4 SR software, from Thermo-Fisher Scientific, Sunnyvale, CA, USA.

2.2.2. LC-DAD-MS

Liquid chromatography coupled with diode-array detection and mass spectrometry (LC-DAD-MS), was used to determine the potential transformation products of all the degradation reactions. The system was composed of a HPLC pump (Finnigan Surveyor LC Pump Plus), an auto-sampler (Finnigan Surveyor Auto-sampler Plus) and a photo-diode array detector equipped with a LightPipe flow cell (Finnigan Surveyor PDA Plus Detector) (all instrumentation was purchased from Thermo Electron Corporation, Waltham, USA), coupled with the same corresponding reverse-phase, silica-based columns discriminated in the previous sub-section. The mass spectrometer was a Finnigan LCQ Deca XP Max, from Thermo Electron Corporation, Waltham, USA, coupled to the LC system and equipped with an electrospray interface as ionization source and a quadrupole ion trap for MSⁿ experiments. The system was operated in positive ion mode between $m/z = 150$ and 1000, with the following conditions: spray voltage, 5.0 kV; capillary voltage, 15 V; and capillary temperature, 300°C. The device was programmed to perform a full mass scan first, and then a fragmentation analysis (MS/MS) of the most intense ion detected. The mobile phases and respective flow-rates, were as described in the previous sub-section, and the mixtures were equally filtered by pressure through the same borosilicate glass funnel (4-8 microns of pore size). The system was also controlled with the Xcalibur 1.4 SR software, from Thermo-Fisher Scientific, Sunnyvale, CA, USA.

2.2.3. Performance of the analytical method

The optimization and performance of the analytical methods for each pharmaceutical compound in particular, were evaluated according to the ICH standard guidelines for validation of analytical procedures [19]. Calibration of the method was carried out separately for each compound and accomplished with the analysis of 9-10 standard solutions between the ranges 0.4984 - 15.00 mg/L (1.682×10^{-6} - 5.062×10^{-5} mol/L) for EE2; 0.04868 - 25.00 mg/L (2.060×10^{-7} - 1.058×10^{-4} mol/L) for CBZ; and 0.05261 -

25.00 mg/L (3.480×10^{-7} - 1.654×10^{-4} mol/L) for PAR. Three trials were made for each solution, yielding three correlation plot lines for each pharmaceutical. Correlation coefficients were all determined above 0.999 for EE2, between 0.996 and 0.999 for CBZ, or all above 0.998 for PAR.

2.3. Disinfection-induced degradation

2.3.1. Determination of kinetic parameters

Experiments were meant to be as simple as possible, so their design reflected such premise. Subsequently, reactions took place in simple 250 mL borosilicate beakers, by using deionised water as a medium, at neutral pH, room temperature, in the dark and with constant stirring.

The first step, was to prepare the stock solutions for the three compounds. The stock solution for PAR was prepared by dissolving 0.7516 g of compound in 100.00 mL of methanol (final concentration being 7516 mg/L or 0.04972 mol/L). The stock solution for EE2 was prepared by dissolving 25.56 mg of compound in 20.00 mL of methanol (final concentration 1278 mg/L or 0.004312 mol/L). As for CBZ, the stock was prepared by dissolving 30.43 mg of pharmaceutical in 25.00 mL of methanol (final concentration 1217 mg/L or 5.151×10^{-3} mol/L).

The initial concentrations of the compounds in the degradation reaction mixtures, were decided to be 2 mg/L for EE2 and PAR, and 0.5 mg/L for CBZ. An important compromise must be achieved in liquid chromatography, when attempting to follow degradation reactions: the compounds' levels must be sufficiently low, in order to be comparable to those occurring in environmental context; additionally, these must also be high enough to enable the accurate following of the reactions by chromatographic analysis. In the case of PAR, a standard solution was always prepared every day of experimentation, from the existing stock, by diluting 100.00 μ L of stock into 10.00 mL of deionised water, with the inclusion of 100.00 μ L of formic acid (when in solution, PAR readily establishes an equilibrium between its ionic and non-ionic forms, and the addition of acid induces the equilibrium to shift towards the predominance of one form). The final concentration of these standards was 75.16 mg/L or 4.972×10^{-5} mol/L. The PAR levels in the reaction mixtures were assured by transferring the appropriate volume from these standard solutions. As for CBZ, the procedure was similar: standard

solutions of CBZ were also prepared every day of experimentation, by diluting 500.00 μL of stock into 10.00 mL of methanol (final concentration 60.85 mg/L or 2.575×10^{-4} mol/L). CBZ levels in the reaction mixtures were also assured by transference of the adequate volume from these standards. In the case of EE2, no standard solutions were prepared prior to the degradation studies, and the concentration of the compound in the reaction mixtures was assured by transferring the correct volume from the stock solution. In summary, reactions were carried out in vessels containing a specific concentration of a single pharmaceutical (2.000 mg/L or 1.323×10^{-6} mol/L in the case of PAR; 0.5002 mg/L or 2.117×10^{-6} mol/L in the case of CBZ; 1.994 mg/L or 6.727×10^{-6} mol/L in the case of EE2), and 5.002 mg/L (or 6.717×10^{-5} mol/L) of NaOCl, in 100 mL of deionised water. This context ensured, in theory, that the variance in NaOCl levels would remain negligible throughout the degradation reactions. [Table 2](#) presents a compilation of the experimental conditions and the monitoring of these throughout the kinetic experiments.

Prior to the addition of NaOCl into the water solution containing solely the pharmaceutical, an aliquot would always be analysed by HPLC-UV-DAD, in order to obtain a chromatographic record of the initial amount of compound. After this step, active chlorine would be added to the solution, pH monitored and regulated, if necessary, and the water temperature recorded and also monitored throughout the experiment. After preliminary assessment of the reactivity of each compound towards active chlorine, and conclusions drawn as to their potential for degradation, a reaction time was determined for each compound. EE2 was concluded to be the most reactive, so a reaction time window around 90 seconds was considered enough, with the removal of an aliquot every 10 seconds. PAR was considered to be slightly more stable, so a reaction time window of about 60 minutes was applied, with aliquots being removed every 10 minutes. As for CBZ, it was regarded as the most stable of the three, so a reaction time window of 360 minutes was considered appropriate, with aliquots being taken at each hour.

2.3.2. Identification of DBPs

The experimental conditions were similar to those described in the previous subsection. In this case, the reactions were left undisturbed for 60-120 minutes, and after this period of time, the entire reaction mixtures were pre-concentrated by means

of SPE (by using 6 mL, 500 mg, hydrophilic-lipophilic balance cartridges from Waters Corporation, Milford, MA, USA). Before the procedure itself, the cartridges were always conditioned with 3 mL of methanol and 3 mL of deionised water. This process was enhanced with the aid of a peristaltic pump (Minipuls® 2 from Gilson, Inc., Middleton, WI, USA). The analytes of interest, adsorbed in the cartridge filter, were then extracted with 3 mL of dichloromethane, subsequently evaporated with a gentle stream of nitrogen gas. The final residue was diluted in 500 µL of methanol and analysed directly by LC-DAD-MS, using the same chromatographic conditions earlier described.

2.3.2. Influence of external variables on degradation

When in the superficial, natural, aquatic compartments, the fate and behaviour of pharmaceuticals are influenced by several external variables, such as active chlorine levels, water pH, water temperature and the presence of DOM in solution as well as its levels. In order to ascertain the degree and extension of influence of these factors on the degradation of the selected pharmaceuticals, an experimental design formulation was created, with statistical analysis of variance (ANOVA) and response surface methodology. This approach, which enabled the determination and outline of tendencies within the results, was processed with the help of the Unscrambler® v9.2 (CAMO Process AS, Oslo, Norway) software program.

These studies were based on two Box-Behnken designs, one with and another without the inclusion of dissolved organic matter (DOM) as a design variable: set 1 (without DOM in solution) and set 2 (with DOM in solution). The designs' features and parameters, are included in [Figure 2](#). [Tables S1-S2](#) include the complete experimental conditions for both designs.

2.4. Photo-induced degradation

Natural photo-degradation is viewed as an important factor for the mitigation of pharmaceuticals in the natural, aquatic, environmental compartments, but artificial mechanisms are also implemented in water treatment facilities, in order to enhance degradation and removal of these compounds from the water courses [7]. However, both natural and artificial photo-irradiation seem to have a tenuous and rather slow effect on degradation, yielding varying degradation rates to the point of being

recognized as not very relevant [7]. In light of these considerations, it was important to test the stability of the considered pharmaceuticals towards artificial photo-irradiation.

Artificial irradiation was performed by means of a solar radiation simulator (Newport Oriel 96000 150 W), with Air Mass Filters 1.5 Global 81094 and a liquid light guide. The latter was always positioned about 1 centimetre above the surface of the aqueous solution.

The general outline of the experimental conditions, was similar to what was described in section 2.3.1, although no active chlorine was ever used or applied in the experiments and so the solutions exposed to artificial solar radiation included only the individual pharmaceuticals. New standard solutions of these compounds were always prepared prior to the experiments, just as described in section 2.3.1. Similarly, photo-degradation reactions took place in simple borosilicate beakers, in 100 mL of deionised water spiked only with the individual pharmaceuticals, and with constant stirring. Initial concentrations of the compounds, prior to irradiation, were 2.000 mg/L (or 1.323×10^{-6} mol/L) for PAR, 0.5002 mg/L (or 2.117×10^{-6} mol/L) for CBZ, and 1.994 mg/L (or 6.727×10^{-6} mol/L) for EE2. HPLC-UV-DAD and UV-Vis spectrophotometer analysis of the solutions, were carried out, before initiating the irradiation process, in order to record the initial conditions of the solutions or compounds. Photo-degradation reactions lasted for a period of 6 hours, with an aliquot being removed, and analysed by HPLC-UV-DAD and in the UV-Vis spectrophotometer, at each period of 60 minutes.

3. Results and discussion

3.1. Disinfection-induced degradation

3.1.1. Determination of kinetic parameters

According to a comprehensive review on the chlorination reactions of organic and inorganic compounds in aqueous solution [20], organic compounds such as pharmaceuticals commonly react with active chlorine in a global second-order kinetic delineation. In other words, first-order regarding the concentration of organic compound and first-order in regards to the total active chlorine in solution. And so, for

the reaction between each pharmaceutical and active chlorine in aqueous solution, we will have:



$$v = \Delta[\text{Products}]/\Delta t = -\Delta[\text{Pharmaceutical}]/\Delta t = -\Delta[\text{Cl}_2]/\Delta t = k_{\text{app}}[\text{Pharmaceutical}][\text{Cl}_2]_t$$

The variable $[\text{Pharmaceutical}]$ regards the concentration of compound, whereas $[\text{Products}]$ represents the concentration of the compounds formed in the reaction. The variable $[\text{Cl}_2]_t$ represents the total concentration of active chlorine ($\text{ClO}^- + \text{HClO}$). The presence and predominance of these active species is strongly pH-dependent [20]. The term k_{app} refers to the apparent global second-order reaction rate constant. Applying a pseudo-first-order experimental scenario to the reaction, we can simplify the kinetic law in the following manner:

$$[\text{Cl}_2]_t \gg [\text{Pharmaceutical}] \Leftrightarrow \Delta[\text{Cl}_2]_t \approx 0$$

$$v = -\Delta[\text{Pharmaceutical}]/\Delta t = k_{\text{obs}}[\text{Pharmaceutical}]$$

Where the variable k_{obs} represents the effectively observed pseudo-first-order rate constant of the studied reaction ($k_{\text{obs}} = k_{\text{app}}[\text{Cl}_2]_t$).

Figure 3 presents the exponential plot of the relative concentration of each pharmaceutical versus the corresponding reaction time. Table 3 includes the compilation of all the relevant kinetic data obtained in the study of the degradation of each compound with active chlorine. Results from Figure 3 and Table 3 portray a striking difference in reactivity between the three compounds. CBZ was clearly the most stable in exposure to active chlorine ($t_{1/2} = 481.35 \pm 16.71$ minutes), achieving a degradation rate of merely 40% after 6 hours of reaction time. PAR was considerably more reactive ($t_{1/2} = 113.63 \pm 3.73$ minutes), displaying a little over 30% degradation rate in 60 minutes. In the case of EE2, however, the compound proved to be overwhelmingly more reactive than the previous ones ($t_{1/2} = 38.59 \pm 1.93$ seconds), presenting a nearly 80% degradation rate in just 70 seconds of reaction time. The molecular structure of the compounds may play a significant factor in their striking differences in reactivity. CBZ displays three unsaturated rings with considerable electronic delocalization throughout the molecule, which grants the compound significant overall stability. PAR, on the other hand, presents a more ordinary structure

to common organic pollutants, with an unsaturated benzenic ring and two particular functional groups that affect its reactivity: the hydroxyl (-OH) functional group is highly *ortho*- and *para*- activating or director, which means that active chlorine will substitute essentially on those positions in relation to said group; however, in the *para*- position is yet another *ortho*- and *para*- activating group, a secondary amine (-NHCOCH₃), although in this case, the amine is a moderately activating group. The presence of two *ortho*- and *para*- activating functional groups in the molecule, in addition to the fact that both groups are electron donors, may explain its lower stability and therefore higher reactivity towards active chlorine. On the other hand, the presence of these *ortho*- and *para*- activating groups in symmetrical positions to one another, may therefore contribute to a slight cancelling or opposing effect, which may explain why PAR is not so reactive as EE2, for instance. Finally, in regards to EE2, the compound displays a larger structure with two hydroxyl groups, which may enhance active chlorine attack on the molecule and therefore explain its considerable reactivity.

3.1.2. Determination of DBPs

Figures 4, 5 and 6 present the LC-DAD-MS spectra for the individual degradation reactions of the three pharmaceuticals, PAR, CBZ and EE2, together with the mass spectra of the identified compounds. Additional data on the identification of the compounds is provided in Table 3, with retention times, most prominent fragmentation ions and estimated molecular formulas. Other data will be compiled in section 1 of the supplementary material, namely all the mass spectra of the compounds and subsequent fragmentations, in Figures S1-S15.

As visible from the analysis of the data in Figure 4 and Table 4, the degradation reaction of PAR with active chlorine yielded two distinct monochlorinated DBPs, Cl-PAR1 and Cl-PAR2. According to the literature [20], chlorination of organic aromatic compounds takes place on the benzenic ring, and specifically around *ortho*- and *para*-activating functional groups. From the analysis of its molecular structure presented in Figure 1, PAR has two *ortho*- and *para*- activating functional groups positioned in opposite ends of the molecule, namely the -OH and -NHCOCH₃. Despite being both *ortho*- and *para*- directors, the hydroxyl group is a very strong activator whereas the secondary amine is merely a moderate activator. Subsequently, the chlorine attack on the molecule, may have taken place more likely at both the *ortho*- positions in regards

to the hydroxyl group, therefore yielding the two monochlorinated by-products. No dichlorinated species was detected in the analysis. Regarding the MS results, analysis in positive mode of the three peaks highlighted in [Figure 4](#), led to the successful identification of the parental compound and the two dichlorinated DBPs: peak at 10.04 minutes yielded an m/z value of 152.40, consistent with the molecular mass of PAR, 151.1626 g/mol; and peaks at 19.06 and 20.07 originated the m/z values 186.53 and 186.47, both consistent with the molecular mass of monochlorinated PAR, 185.6074 g/mol.

In the case of CBZ, its degradation reaction was reported in the previous section as the slowest of the compounds considered in this study, as visible in [Figure 3](#), and also fittingly, it originated merely one DBP, the monochlorinated analogue of CBZ, Cl-CBZ. Analysis of the reaction solution by LC-DAD-MS, allowed the successful identification of the parental compound as well as the monochlorinated structure: peak at 13.78 minutes was determined to represent the parental compound, CBZ, yielding an m/z value of 237.16, consistent with its molecular mass, 236.2687 g/mol; and the peak at 18.06 minutes was identified as the monochlorinated DBP of CBZ, Cl-CBZ, originating an m/z value of 271.80, which is consistent with the corresponding molecular mass, 270.7214 g/mol. CBZs molecular structure has two benzenic rings coupled together through another cyclic structure. In the overall structure, an amine is connected or linked to both benzenic rings, and amines are considered moderate to strong *ortho*- and *para*- activators. Subsequently, the active chlorine electrophilic substitution will most likely take place at the *ortho*- position to the $-NR_2$ group atom, in either of the two benzenic rings. It is also important to underline the fact that the most prominent CBZ fragment ions ($m/z = 194.31$ and 219.99), also occurred in the case of the DBP ($m/z = 193.33$ and 219.93), which demonstrates the similarity between the compounds.

Regarding EE2, its degradation reaction was incredibly fast, as reported in the previous section, with 80% of it being degraded in just over one minute. In these experiments, however, the reactions were allowed to run their course, so the degradation of EE2 was also always complete, reason why no parental compound was ever detected in the analysis of the reaction mixtures by LC-DAD-MS. To circumvent this issue, direct injection by MS was performed, prior to the reaction, in order to obtain its mass spectrum and fragmentation profile (see supplementary material): the compound was detected with the m/z value 297.27, which is consistent with its

molecular mass, 296.4034 g/mol. As for the reaction mixture itself, LC-DAD-MS analysis allowed the identification of the compound's mono and trichlorinated DBPs, as visible in [Figure 6](#): peak at 11.77 minutes was identified as the monochlorinated analogue of EE2, Cl-EE2, showing an m/z value of 332.40, consistent with the corresponding molecular mass, 331.8561 g/mol; and the peak at 14.48 minutes, was determined to be the trichlorinated DBP of EE2, the Cl₃-EE2, displaying an m/z value of 399.13, consistent with the molecular mass 399.7377 g/mol.

3.1.3. Influence of external variables on degradation

[Figure 2](#) describes the experimental conditions of the two Box-Behnken designs that were created. As mentioned in section 2.3.2, Set 1 didn't include DOM in solution, whereas Set 2 did. The purpose of this approach, was to ascertain the degree of influence of DOM in the overall degradation rates of the compound, since DOM is known to actively compete with synthetic organic compounds, for the available free chlorine in solution. This degree of influence, not just of DOM but every other variable studied, was evaluated statistically by means of analysis of variance (ANOVA). The ANOVA tables, as well as the response surfaces generated in the study for every pharmaceutical considered in this work, are included in supplementary material. [Tables S3-S8](#), present the ANOVA for every model and every compound, whilst [Figures S16-S21](#) present the response surfaces obtained in those studies.

First, it must be highlighted that both models, Set 1 and Set 2, were statistically validated in each study with every compound, with details being thoroughly discriminated in section 2 of the supplementary material. As far as the results are concerned, mostly the pH and the NaOCl concentration showed significant impact in shaping the degradation rates of PAR, CBZ and EE2. Differences in water temperature did not influence the results in any given way. The presence of DOM in solution, however, did prove meaningful, albeit not at the same level as the pH and active chlorine levels. In fact, results regarding DOM levels showed that their increase led to the decrease in the degradation rates, particularly in the cases of PAR and EE2. From the analysis of [Figures S16-S21](#), one can perceive that the presence of DOM in solution was enough to lower the maximum degradation rates of PAR and EE2 by 20%. Maximum degradation rates of PAR were determined around 73% without DOM, whereas with DOM in solution, maximum values lowered towards 52%. In the case of

EE2, without DOM, maximum degradation was determined at 85%, while the value lowered towards 63% with DOM in solution. These findings in general are consistent with the common knowledge that organic matter dissolved in superficial waters, actively competes with other organic compounds, namely synthetic ones, for the available free chlorine in solution. In the case of CBZ, though, this effect was deemed negligible to irrelevant, since maximum degradation rates did not change and the standard deviation of the variable also proved to be too prominent.

To conclude this matter, PAR degraded more significantly at higher concentrations of active chlorine together with higher values of pH (a context characterized by the predominance of hypochloride anion as the prevailing chlorine disinfecting agent [20]), suggesting that the ionic form of the pharmaceutical also predominates in solution. The lowest degradation rates for PAR, were obtained around the entire scope of active chlorine levels and at lower-medium pH values. Superficial fresh waters usually display pH values around 6-8, depending on the conditions of the soils around and bedrock [23], while seawater displays a pH around 8 [23]. This means that, in these contexts, degradation rates no higher than 40% should be expected for PAR, as indicated in [Figures S16 and S17](#). For CBZ, the highest degradation rates were obtained at higher active chlorine concentrations and lower pH values, as [Figures S18 and S19](#) clearly indicate. These findings mean that the non-ionic form of the compound likely predominates in solution, but also that it is more reactive towards hypochlorous acid, the chlorine species predominant at lower pH and known to be a much more efficient disinfecting agent [20]. CBZ degradation rate was deemed slow and low, around 23-26% at maximum, and these should only occur at deeper and more acidic waters with significant stratification effects [23]. In ordinary superficial fresh water and sea water, CBZ degradation should not be expected to surpass 20%. In the case of EE2, however, maximum degradation was determined at higher concentrations of active chlorine and higher pH values, as visible in [Figures S20 and S21](#). This indicates that the ionic form of the compound is more prominent in solution, and therefore displays more significant reactivity towards hypochloride anion [20]. Regarding EE2, considerable degradation rates should be expected, at ordinary superficial waters, namely around 50-70%.

3.2. Photo-induced degradation

3.2.1. Determination of kinetic parameters and photo-stability assessment

Photo-induced degradation studies were performed in exactly the same manner as the previous studies, although without active chlorine in solution. Experimental conditions for this part of the work, are described in section 2.4. In general, reactions took place in ordinary borosilicate beakers containing 100 mL of deionised water, spiked with the pre-defined amount of pharmaceutical, and in constant stirring. The solutions would then be continuously exposed to artificial solar radiation, with an aliquot being removed at each hour mark, in order to be analysed in the UV-Vis spectrophotometer and by HPLC-UV-DAD. Prior to the exposure of the solution, an aliquot was taken and analysed the same way, in order to obtain a chromatographic and spectroscopic record of the initial conditions. [Figure 7](#) presents the exponential plots of the relative concentrations of pharmaceuticals versus the corresponding irradiation time.

Moving on towards the results, as is visible through the analysis of [Figure 7](#), the three compounds behaved rather similarly towards exposure to artificial solar radiation and degraded according to a first-order kinetic profile. In the case of PAR, the kinetic rate constant determined was $k = 0.00078 \pm 0.00002 \text{ min}^{-1}$, whereas the half-time was $t_{1/2} = 888.65 \pm 22.79$ minutes. For CBZ, the results were $k = 0.00149 \pm 0.00008 \text{ min}^{-1}$, whereas the half-time was $t_{1/2} = 465.20 \pm 24.98$ minutes. As for EE2, the kinetic rate constant was $k = 0.00172 \pm 0.00005 \text{ min}^{-1}$, and the half-time was $t_{1/2} = 402.99 \pm 11.71$ minutes. After the same artificial irradiation time of 360 minutes, PAR degraded a little over 20%, whereas CBZ and EE2 degraded over 40%. As the half-time calculation indicate, PAR is significantly more photo-stable than CBZ and EE2, taking almost 15 hours to achieve half of its initial concentration. In the cases of CBZ and EE2, that time period is shortened to about 6-8 hours. According to studies performed [\[2, 7\]](#), natural solar radiation is an important process for degradation and control of synthetic organic pollutants in natural waters. Similarly, artificial irradiation is commonly incorporated into the water treatment systems and facilities, as a way to enhance the mitigation of pharmaceuticals in the water courses for the same purpose. However, in those same studies, the efficiency of latter is considered variable at best, with degradation or removal rates changing from 30-70%. In parallel to the questionable efficiency of artificial photo-irradiation as a mitigating process, several pharmaceuticals have been found to be extremely photo-stable, both towards natural or artificial solar radiation [\[7\]](#).

One of such pharmaceuticals is PAR, a result which was confirmed by the findings obtained in the present work. Removal rates obtained for compounds such as CBZ, are around 30% [7], which is consistent with the results also obtained in this work.

In light of the results obtained in the artificial photo-degradation studies, these methods have been found to be indeed inefficient in quantitatively removing prominent pharmaceuticals, such as PAR, CBZ and EE2, from superficial waters. When coupled with disinfection-induced degradation, removal rates should be expected to increase. On the other hand, considering how compounds such as CBZ are extremely stable to chlorine-induced disinfection, these methods in conjunction should not be able to effectively remove the compounds entirely from the water courses.

4. Conclusions

In a context of excess active chlorine, EE2 and PAR were considered to be extremely reactive, with EE2 achieving nearly complete degradation after a little over one minute of reaction time. CBZ was considered much more stable, with about 40% degradation rate after 6 hours of reaction time. Upon studying the impact of external variables on the degradation rates of the pharmaceuticals considered in this work, it was found that merely the pH and active chlorine concentration have significant influence in increasing or decreasing compound degradation. The presence of DOM in solution, however, significantly decreased the degradation rates of the compounds, with the exception of CBZ. This finding, correlates with the knowledge that naturally-dissolved organic matter actually competes with synthetic organic compounds for the available active chlorine in solution. In exposure to active chlorine, all pharmaceuticals yielded the formation of DBPs: two monochlorinated analogues of the parental structure, in the case of PAR; one monochlorinated DBP, in the case of CBZ; and a monochlorinated and trichlorinated analogues of the original compound, in the case of EE2. According to the extreme reactivity of EE2 and PAR towards active chlorine, and also the concerning eco-toxicological profiles of chlorinated organic compounds, it is expected that both these pharmaceuticals may pose a significant risk, if present in the superficial water courses in significant concentrations.

As far as the artificial photo-induced degradation studies, all compounds were considered to be stable, with PAR displaying nearly twice the half-life period of both CBZ or EE2. With natural solar radiation being an important method of compound attenuation in superficial waters, artificial irradiation is often incorporated in water treatment, in order to achieve similar results. However, prominent pharmaceuticals are known to exhibit significant stability to artificial photo-degradation, whilst the method itself displays questionable efficiency. When or if chlorine-induced disinfection is coupled with artificial solar irradiation, degradation rates may indeed increase significantly and the compounds may be quantitatively removed from the water courses. In some cases of extreme stability towards degradation in general, with compounds such as CBZ, removal rates may not be significant and the compound may therefore remain in the water in meaningful concentrations.

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(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf). Last accessed on 07/11/2018.

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Figures

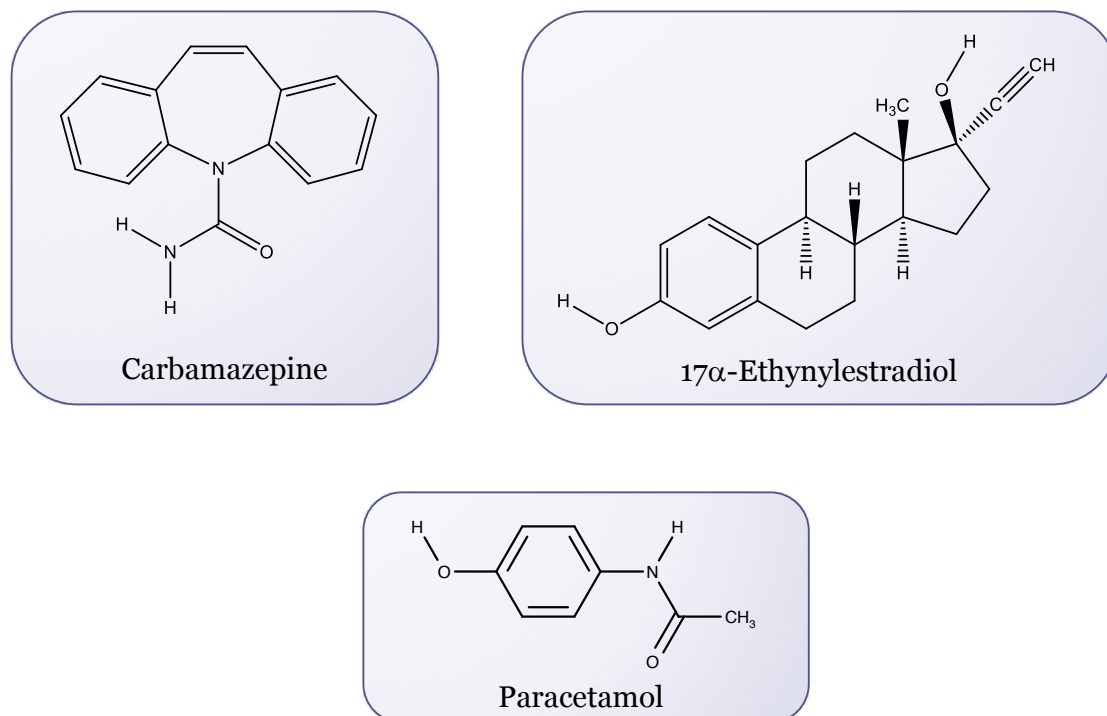


Figure 1. Molecular structures of the compounds addressed in this study (paracetamol or PAR; carbamazepine or CBZ; and 17α-ethynylestradiol or EE2).

- Non-design variable: compound's degradation percentage;
- Design variables: NaOCl levels; pH; temperature;
- Design conditions: 12 experiments (with 2 trials each) + 1 central experiment (with 3 trials) = 27 total experiments;

Set 1

- Non-design variable: compound's degradation percentage;
- Design variables: NaOCl levels; pH; DOM levels; temperature;
- Design conditions: 24 experiments (with 2 trials each) + 1 central experiment (with 3 trials) = 51 total experiments;

Set 2

Figure 2. Detailed description of the experimental condition of the Box-Behnken studies on the influence of external variables on degradation of the compounds.

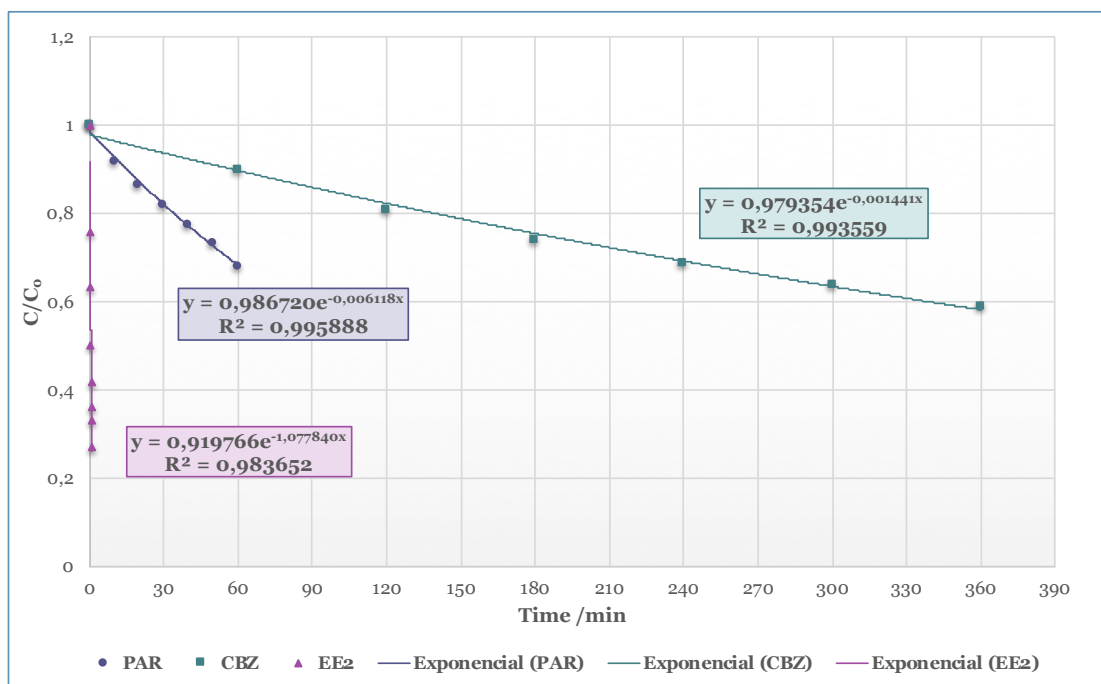


Figure 3. Exponential plot of the relative concentration of pharmaceutical as a function of reaction time.

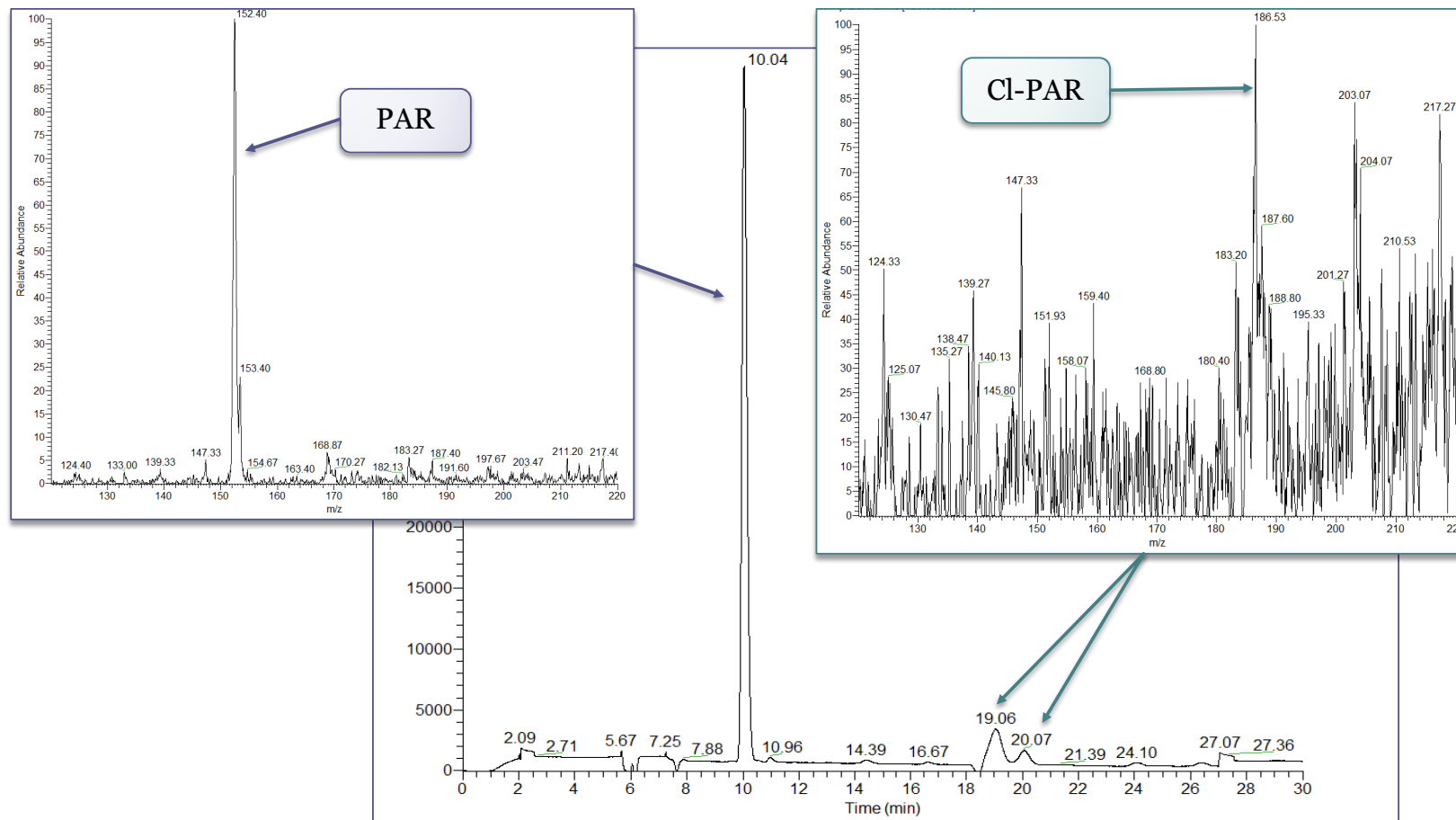


Figure 4. LC-MS-DAD total ion current (TIC) chromatogram of the degradation reaction of PAR with active chlorine, highlighting the peaks successfully identified by MS, and corresponding mass spectra.

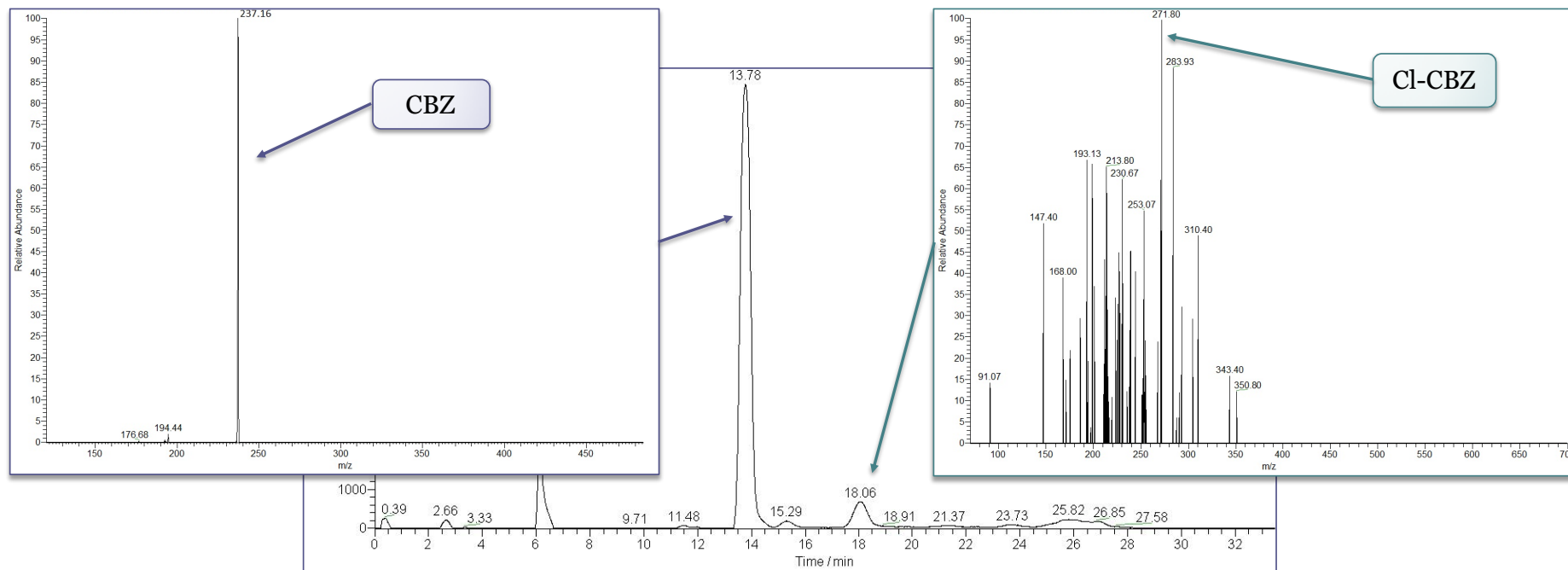


Figure 5. LC-MS-DAD total ion current (TIC) chromatogram of the degradation reaction of CBZ with active chlorine, highlighting the peaks successfully identified by MS, and corresponding mass spectra.

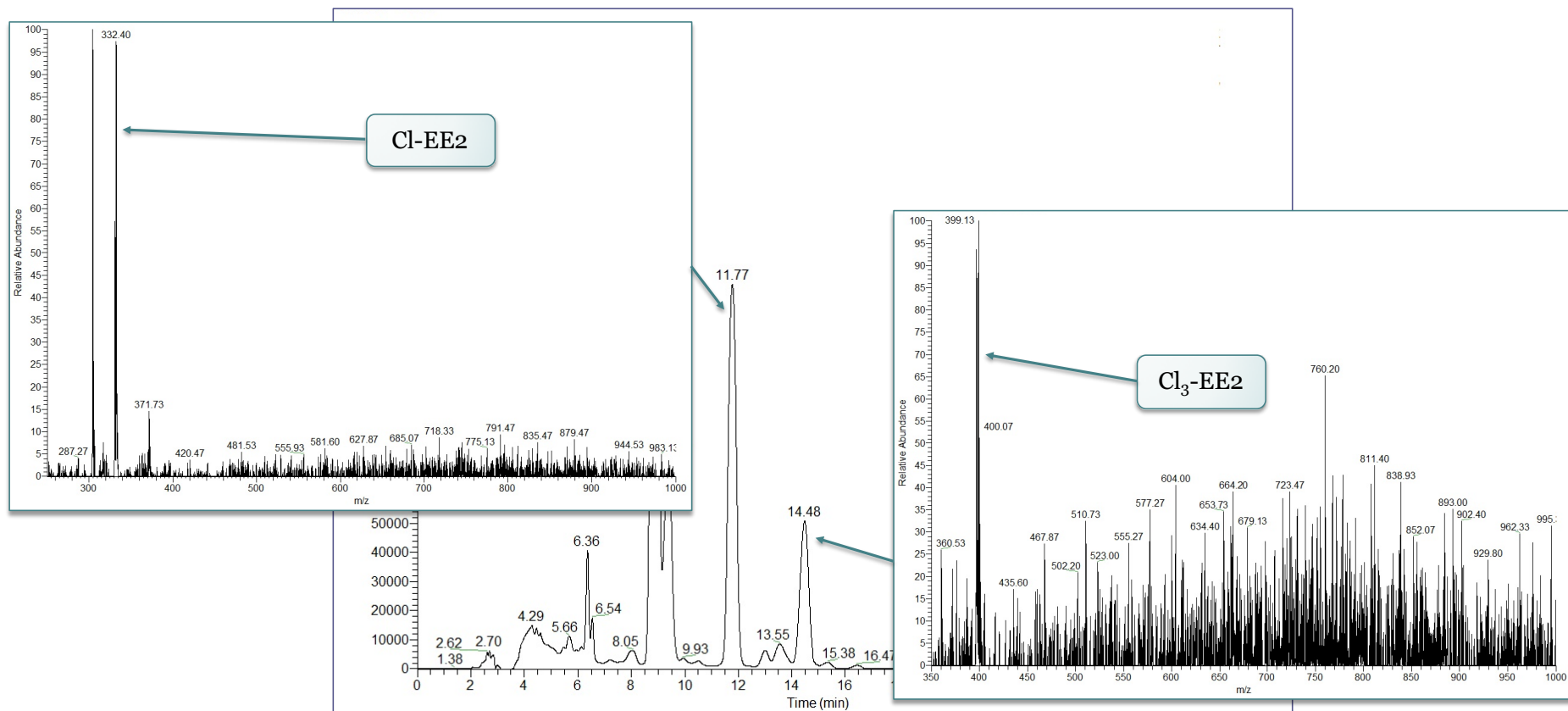


Figure 6. LC-MS-DAD total ion current (TIC) chromatogram of the degradation reaction of EE2 with active chlorine, highlighting the peaks successfully identified by MS, and corresponding mass spectra.

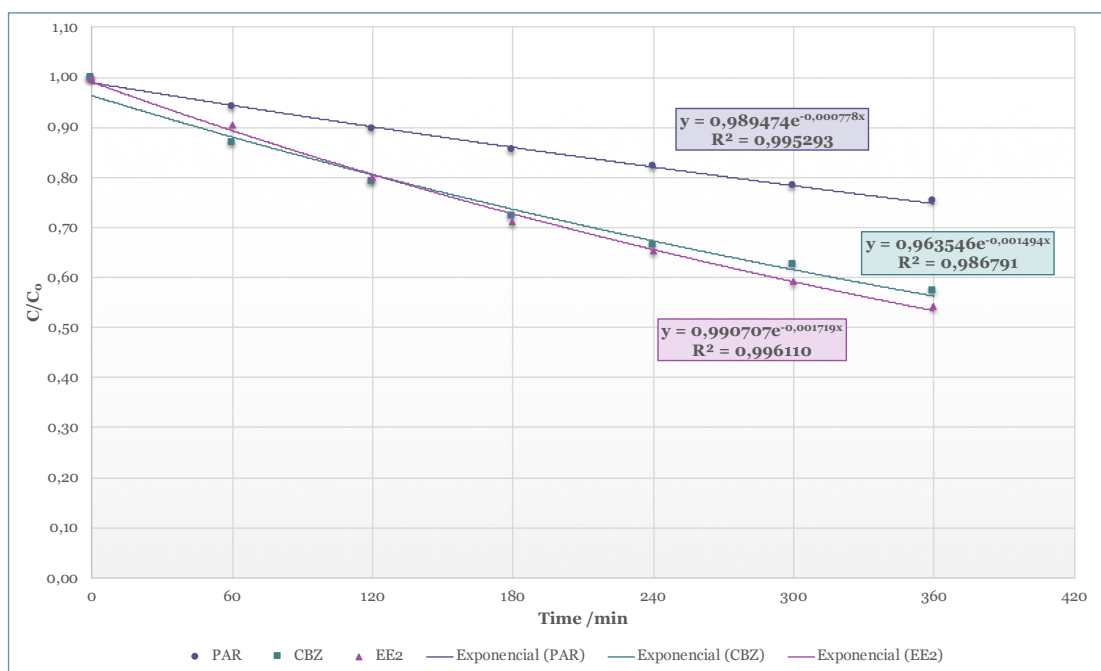


Figure 7. Exponential plot of the relative concentration of pharmaceutical as a function of artificial solar irradiation time.

Tables

Table 1. Physical-chemical properties of the pharmaceuticals considered in this study.

IUPAC name	Acronym	Molecular mass /g/mol	Solubility /mg/L	Log k_{ow}
N-(4-hydroxyphenyl)-acetamide	PAR	151.1626	14000 (in water and at 25°C) *	0.46 *
Benzo[b][1]benzazepine-11-carboxamide	CBZ	236.2687	Very low solubility †	2.45 † ^[21]
(8R, 9S, 13S, 14S, 17R)-17-ethynyl-13-methyl-7, 8, 9, 11, 12, 14, 15, 16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol	EE2	296.4034	11.3 (in water and at 27°C) ‡	3,67 ^[22]
* From the Toxicology Data Network (TOXNET), U.S. National Library of Medicine (https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+103-90-2).				
† From the Toxicology Data Network (TOXNET), U.S. National Library of Medicine (https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+298-46-4).				
‡ From the Toxicology Data Network (TOXNET), U.S. National Library of Medicine (https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+57-63-6).				

Table 2. Experimental conditions for the kinetic studies of each pharmaceutical.

Pharmaceutical	Initial levels	NaOCl concentration	pH	Temperature
PAR	2.000 mg/L or 1.323x10 ⁻⁶ mol/L	5.002 mg/L or 6.717x10 ⁻⁵ mol/L	6.89 ± 0.05	18.4 ± 0.8°C
CBZ	0.5002 mg/L or 2.117x10 ⁻⁶ mol/L		7.23 ± 0.08	19.2 ± 0.9°C
EE2	1.994 mg/L or 6.727x10 ⁻⁶ mol/L		7.12 ± 0.04	18.9 ± 0.6°C

Table 3. Kinetic data obtained from the disinfection-induced degradation reactions of each pharmaceutical.

Pharmaceutical	Mean correlation coefficient (R^2)	Kinetic rate constant (k_{obs})	Half-life period ($t_{1/2}$)
PAR	0.995888	0.0061 ± 0.0002 min ⁻¹	113.63 ± 3.73 min
CBZ	0.993558	0.00144 ± 0.00005 min ⁻¹	481.35 ± 16.71 min
EE2	0.983638	0.0180 ± 0.0009 s ⁻¹	38.59 ± 1.93 s

Table 4. LC-DAD-MS data on all the compounds successfully identified in this study.

Pharmaceutical	Retention time /min	Identification	Molecular ion /m/z	Fragmentation ions /m/z (relative intensity /%)	Proposed molecular formula	Abbreviation
PAR	10.04	[PAR-H] ⁺	152.40	110.10 (100); 134.78 (22);	C ₈ H ₁₀ NO ₂ ⁺	PAR
	19.06	[Cl-PAR-H] ⁺	186.53	166.87 (100); 168.93 (94); 143.98 (77); 153.16 (54); 172.92 (52)	C ₈ H ₉ NO ₂ Cl ⁺	Cl-PAR1
	20.07	[Cl-PAR-H] ⁺	186.47	167.13 (100); 144.05 (66); 168.86 (42); 152.96 (34); 74.00 (35); 172.72 (18);	C ₈ H ₉ NO ₂ Cl ⁺	Cl-PAR2
CBZ	13.78	[CBZ-H] ⁺	237.16	194.31 (100); 219.99 (20);	C ₁₅ H ₁₃ N ₂ O ⁺	CBZ
	18.06	[Cl-CBZ-H] ⁺	271.80	193.33 (100); 236.20 (64); 219.93 (40);	C ₁₅ H ₁₂ N ₂ OCl ⁺	Cl-CBZ
EE2	-	[EE2-H] ⁺	297.27	183.27 (100); 258.07 (90); 200.07 (74)	C ₂₀ H ₂₅ O ₂ ⁺	EE2
	11.77	[Cl-EE2-H] ⁺	332.40	240.33 (100)	C ₂₀ H ₂₄ O ₂ Cl ⁺	Cl-EE2
	14.48	[Cl ₃ -EE2-H] ⁺	399.13	238.93 (64)	C ₂₀ H ₂₂ O ₂ Cl ₃ ⁺	Cl ₃ -EE2

Supplementary material

Fate and behaviour of prominent pharmaceuticals, paracetamol, 17 α -ethynylestradiol and carbamazepine, in aqueous solution

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1. Identification of DBPs

The mass spectra for all compounds, as well as their fragmentations, are presented in this supplementary document, in [Figures S1-S16](#). Identification details will follow. In the case of PAR:

- Peak at 10.04 minutes in [Figure 4](#) of the manuscript (identified as the parental compound, PAR), generated a mass spectrum with an m/z value of 152.40 (100%), which is consistent with its molecular mass (151.1626 g/mol);
 - Fragmentation of the peak yielded fragment ions of m/z values 110.10 (100%) and 134.78 (22%), as the most prevalent;
- Peak at 19.06 minutes in [Figure 4](#) (identified as the first monochlorinated by-product of PAR), generated a mass spectrum with an m/z value of 186.53 (100%), consistent with the corresponding molecular mass (185.6074 g/mol);
 - Fragmentation of the peak led to fragment ions of m/z values 166.87 (100%), 168.93 (94%), 143.98 (77%), 153.16 (54%) and 172.92 (52%), as the most abundant;
- Peak at 20.07 minutes in [Figure 4](#) (identified as the second monochlorinated by-product of PAR), yielded the m/z value of 186.47 (100%), once more consistent with the molecular mass (185.6074 g/mol);
 - Fragmentation of the peak originated the fragment ions 167.13 (100), 144.05 (66), 168.86 (42) and 152.96 (34), as the most prominent;

In regards to CBZ:

- Peak at 13.78 minutes in [Figure 5](#) (identified as the original compound, CBZ) originated the m/z value 237.16 (100%), consistent with the molecular mass of the compound (236.2687 g/mol);
 - Fragmentation of this peak yielded the fragment ions 194.31 (100%) and 219.99 (20%), as the most prevalent;
- Peak at 18.06 minutes in [Figure 5](#) (identified as the monochlorinated by-product of CBZ), generated the m/z value 271.80, which is consistent with the compound's molecular mass (270.7214 g/mol);
 - Fragmentation of the peak led to the ions with m/z 193.33 (100%), 236.20 (64%) and 219.93 (40%), as the most abundant;

As far as EE2 is concerned:

- EE2, being completely degraded in the reaction with active chlorine, was determined by direct injection in mass spectrometry, yielding the m/z value 297.27 (100%), which is also consistent with its molecular mass (296.4034 g/mol);
 - Fragmentation of the compound, led to the detection of the ions 183.27 (100%), 258.07 (90%) and 200.07 (74%), as the most prominent fragments;
- Peak at 11.77 minutes in [Figure 6](#) (identified as the first monochlorinated DBP of EE2), yielded the m/z value of 332.40, consistent with the corresponding molecular mass (331.8561 g/mol);
 - Fragmentation of this peak, originated the ion of m/z 240.33 (100%), as the most relevant;
- Peak at 14.48 minutes in [Figure 6](#) (identified as the trichlorinated by-product of EE2), generated the m/z value of 399.13, consistent with the corresponding molecular mass of the compound (399.7377 g/mol);
 - Fragmentation of this peak, led to the ion with m/z value 238.93 (64%), as the most relevant fragment;

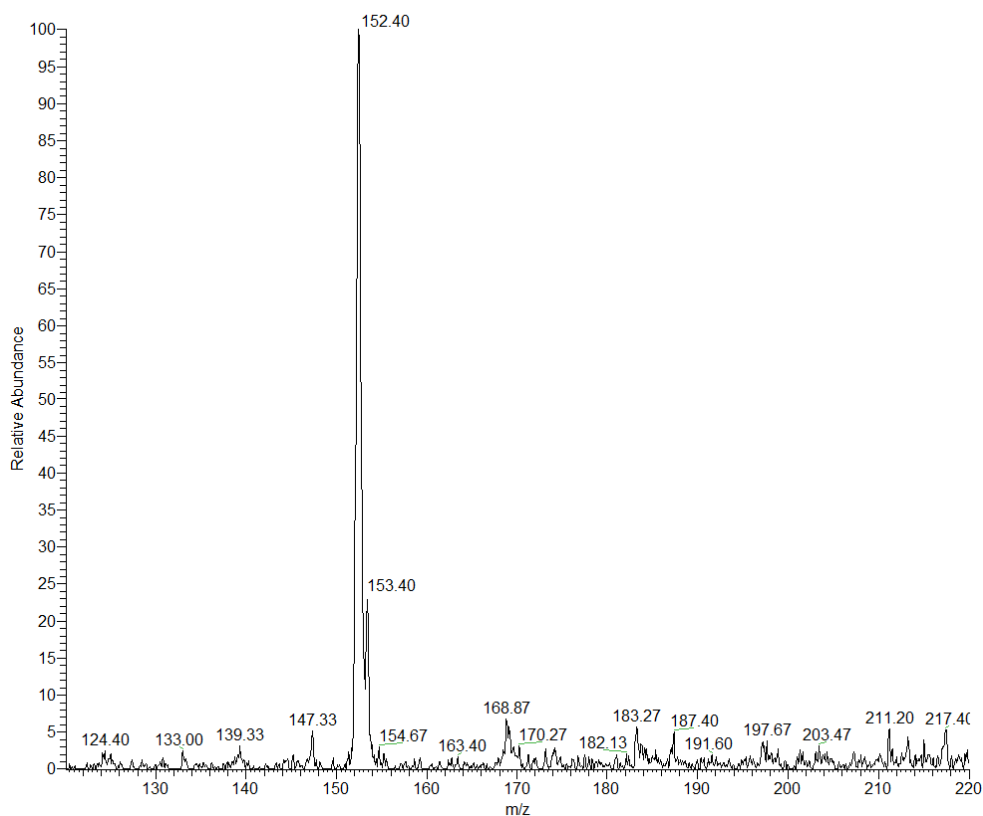


Figure S1. Mass spectrum obtained for PAR.

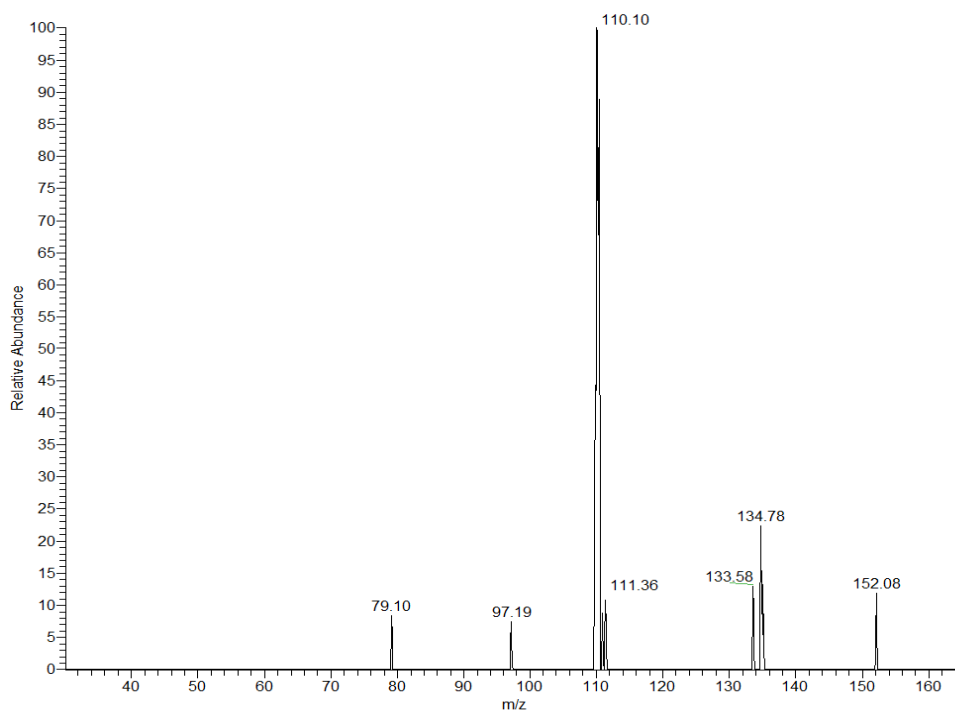


Figure S2. Mass spectrum of the fragmentation of PAR.

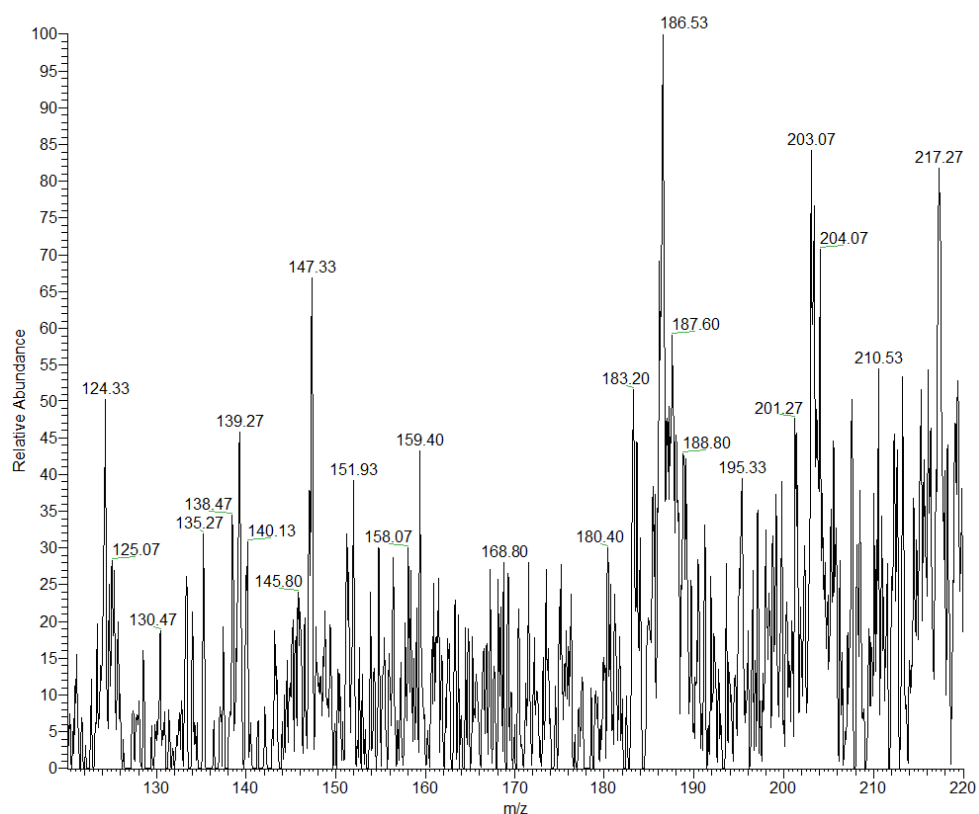


Figure S3. Mass spectrum of the first monochlorinated by-product of PAR, Cl-PAR1.

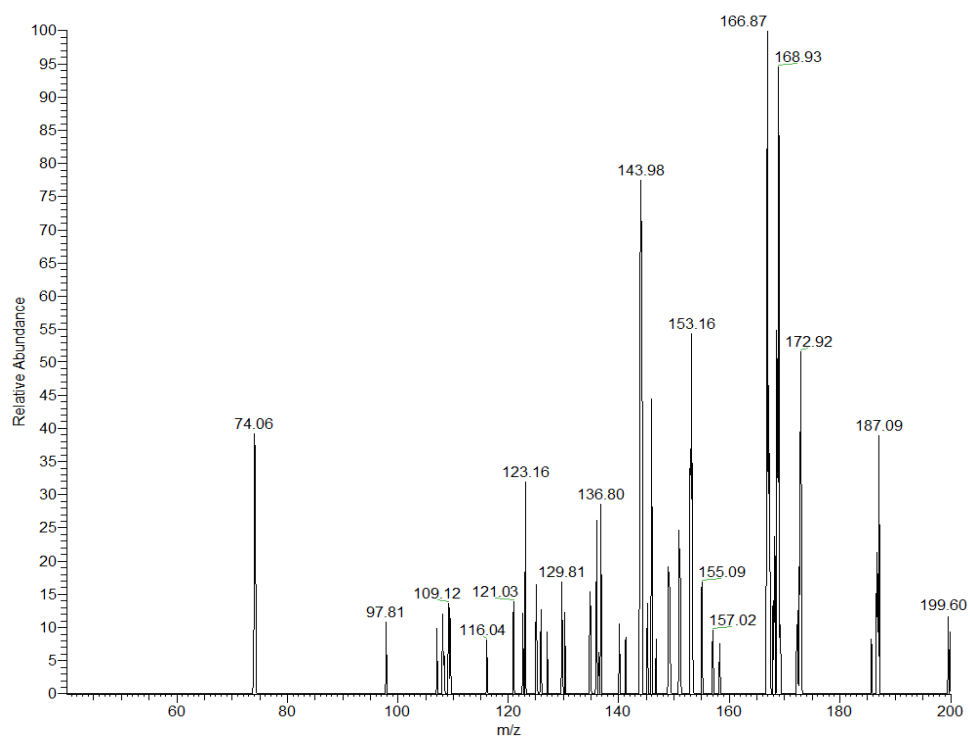


Figure S4. Mass spectrum of the fragmentation of Cl-PAR1.

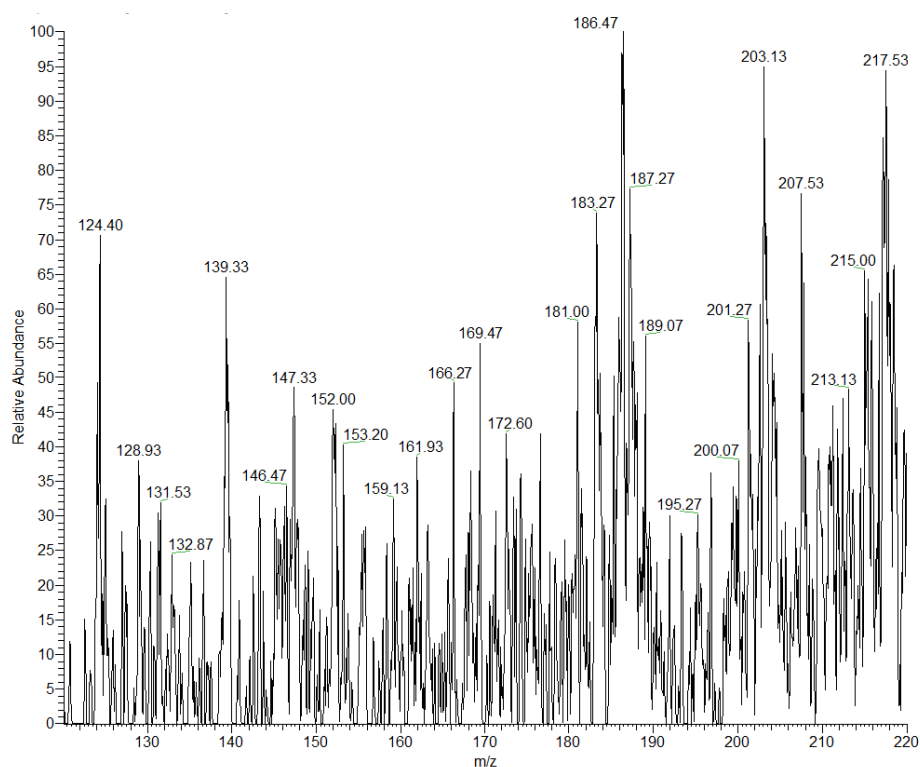


Figure S5. Mass spectrum of the second monochlorinated by-product of PAR, CI-PAR2.

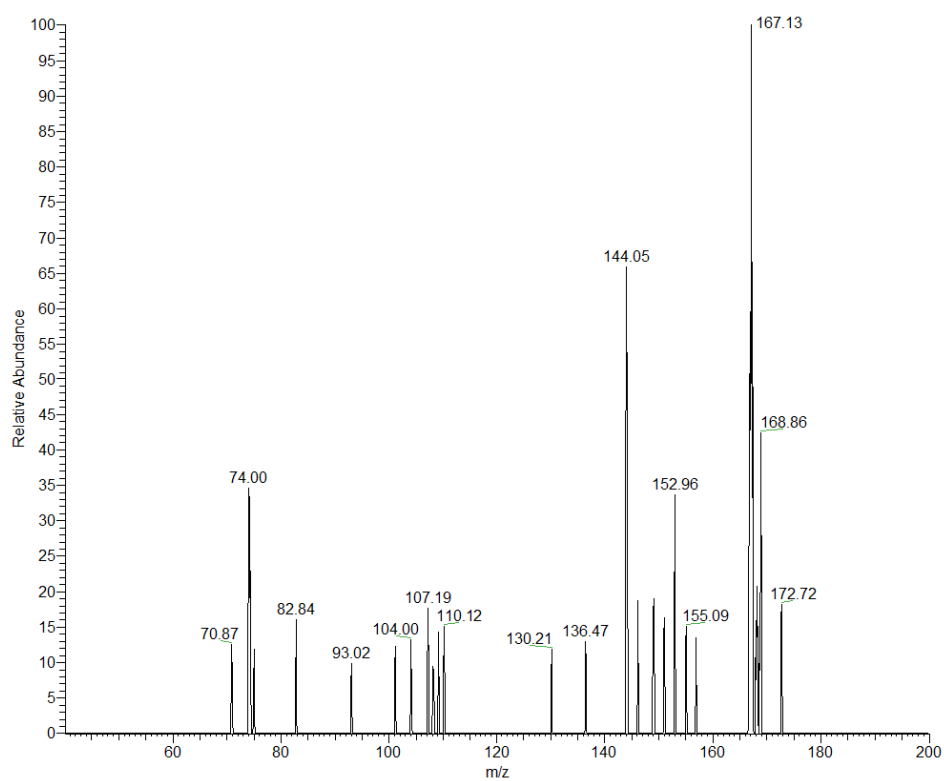


Figure S6. Mass spectrum of the fragmentation of CI-PAR2.

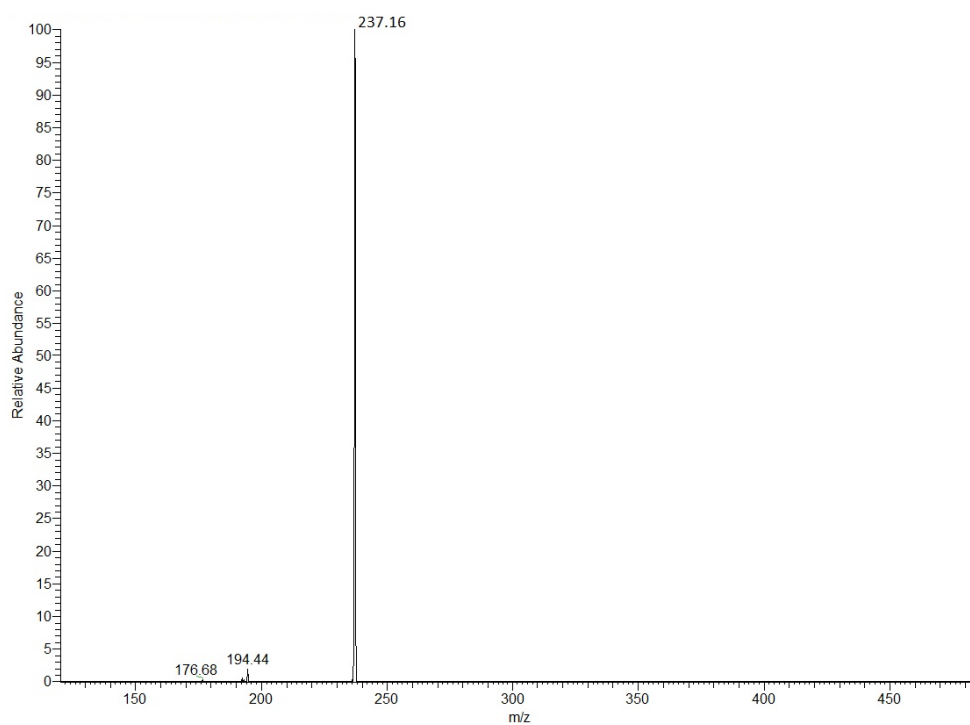


Figure S7. Mass spectrum of CBZ.

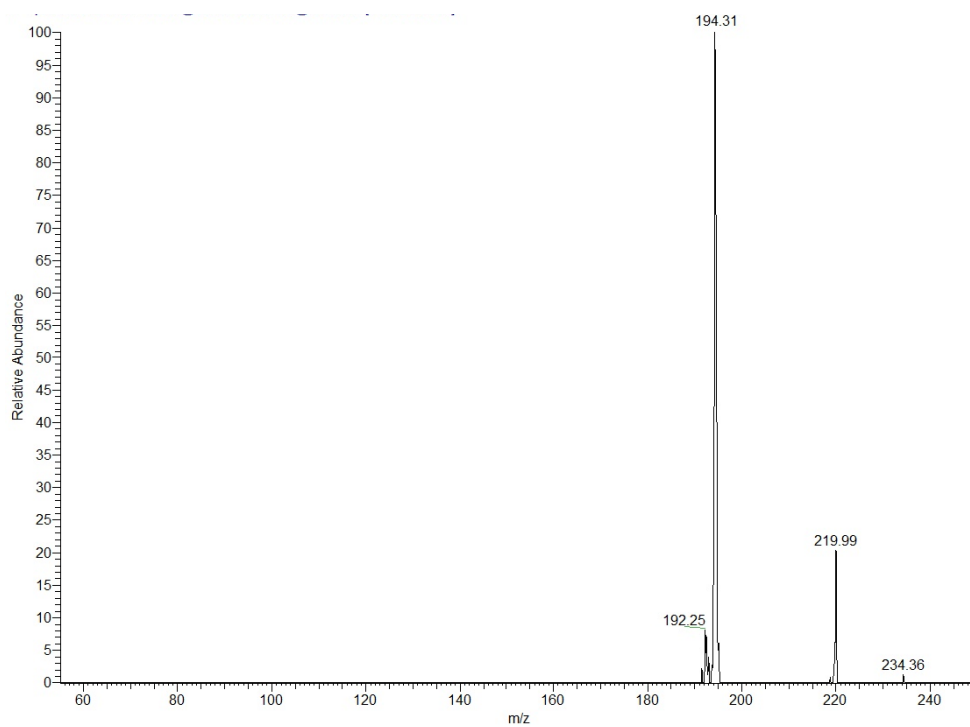


Figure S8. Mass spectrum of the fragmentation of CBZ.

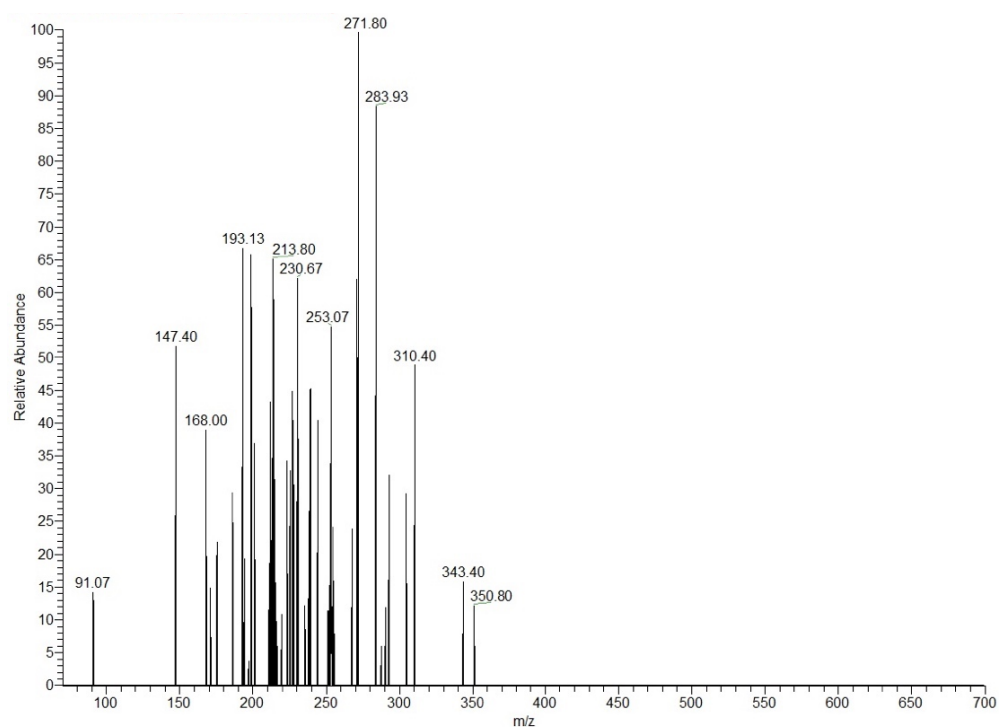


Figure S9. Mass spectrum of the monochlorinated by-product of CBZ, Cl-CBZ.

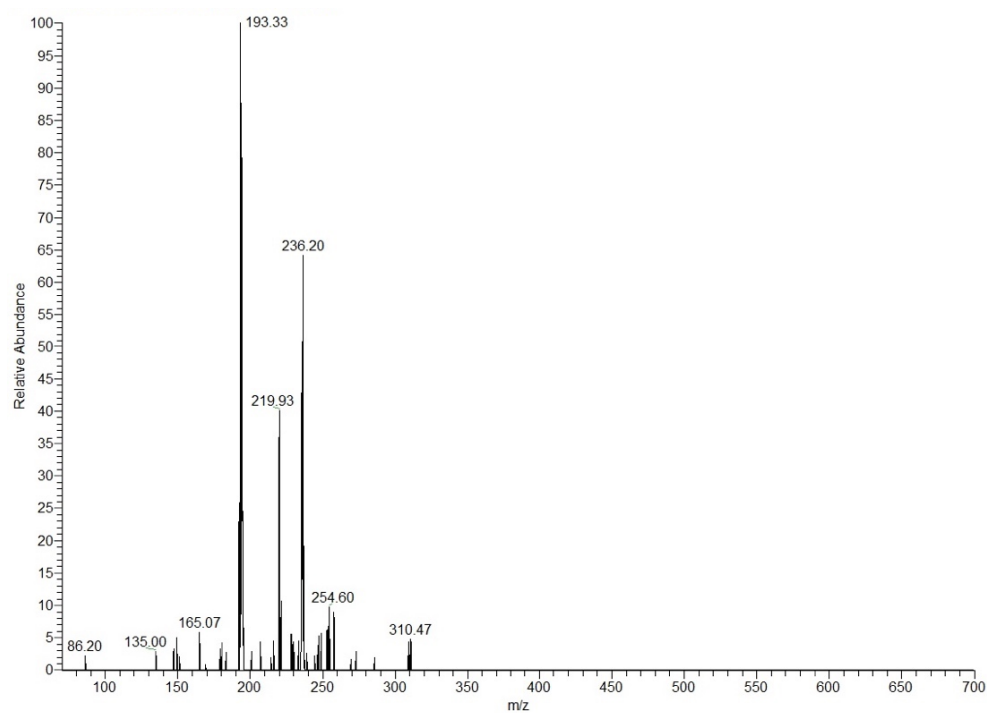


Figure S10. Mass spectrum of the fragmentation of Cl-CBZ.

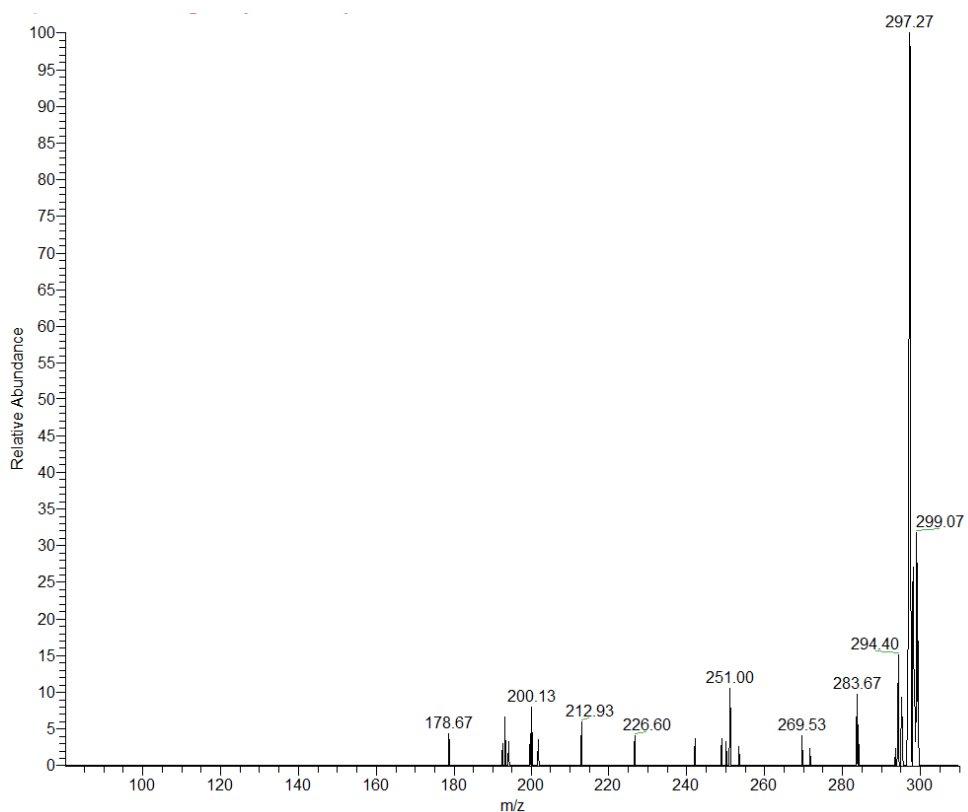


Figure S11. Mass spectrum of EE2.

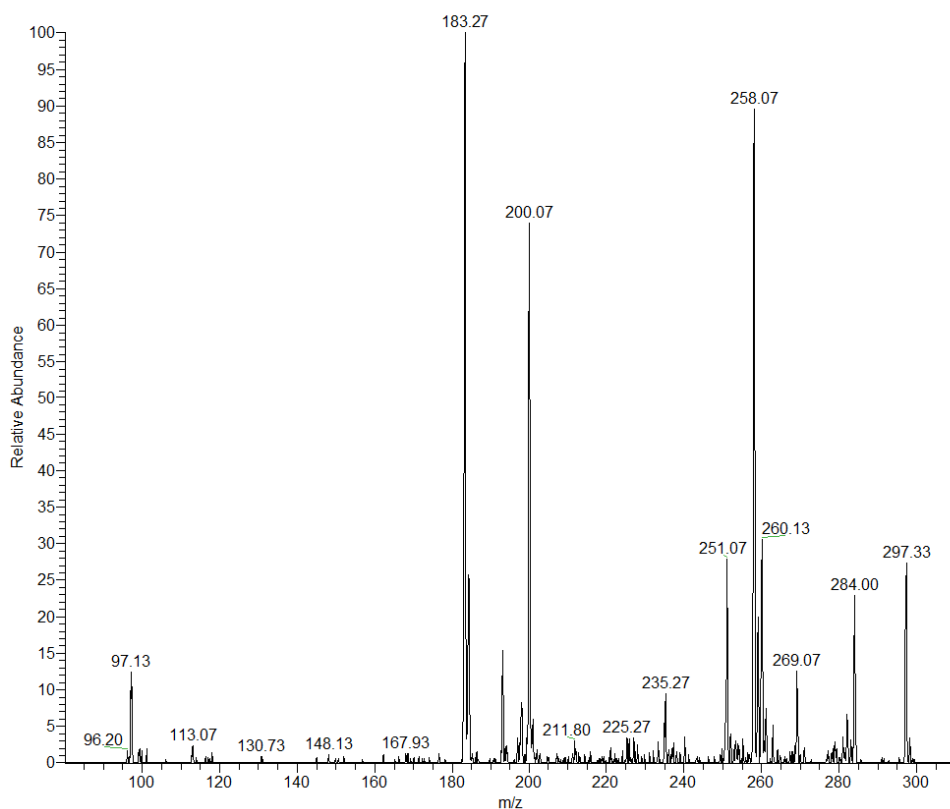


Figure S12. Mass spectrum of the fragmentation of EE2.

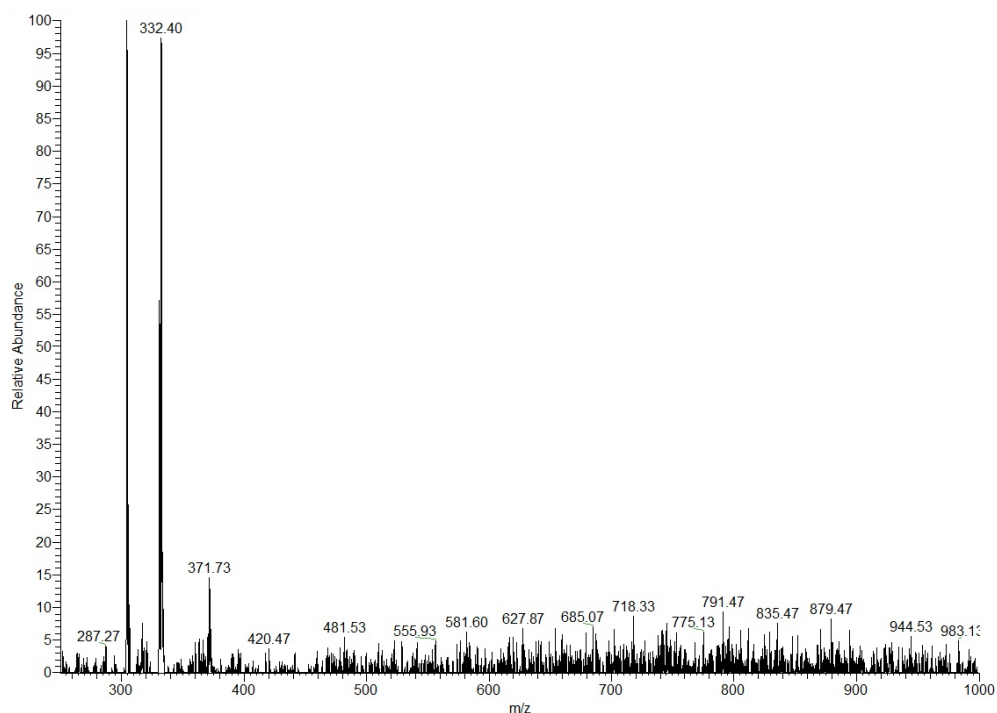


Figure S13. Mass spectrum of the monochlorinated by-product of EE2, Cl-EE2.

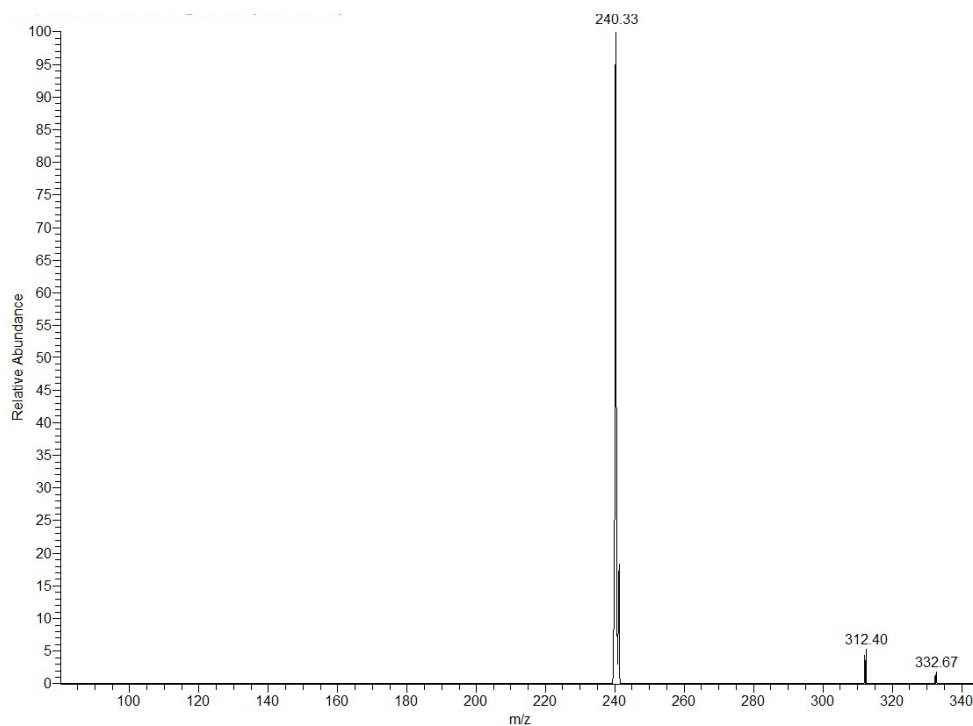


Figure S14. Mass spectrum of the fragmentation of Cl-EE2.

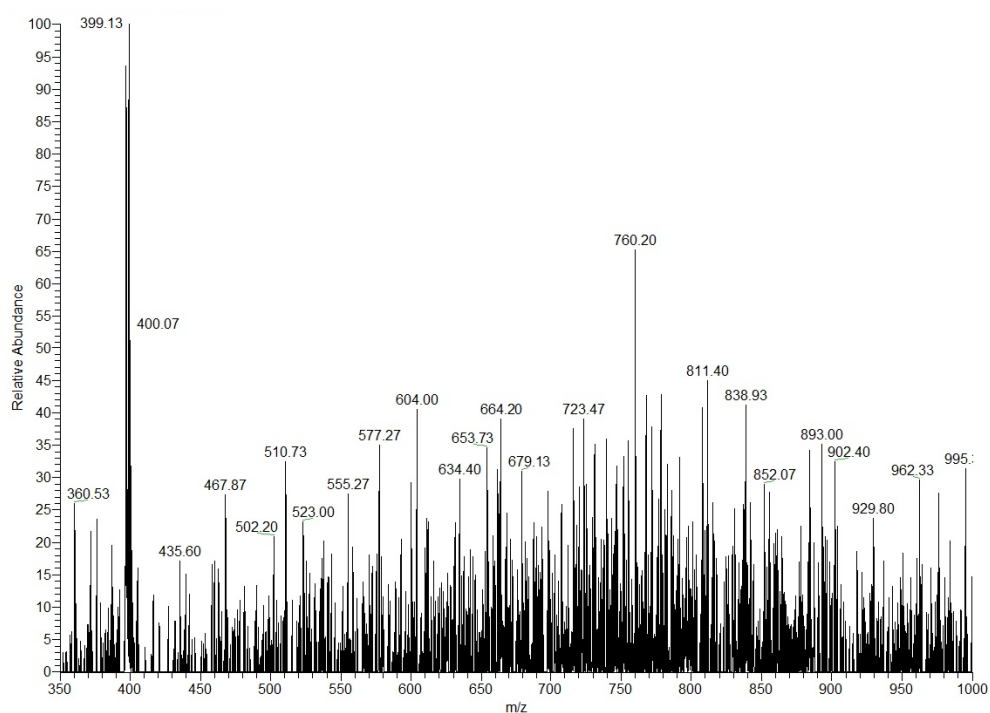


Figure S15. Mass spectrum of the trichlorinated by-product of EE2, Cl₃-EE2.

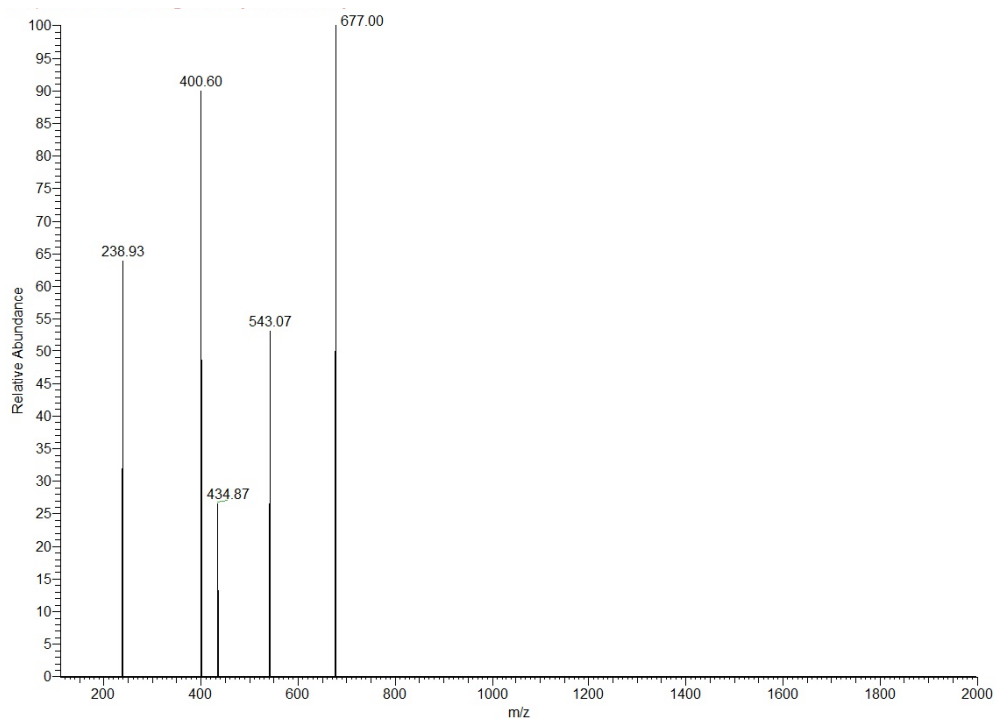


Figure S16. Mass spectrum of the fragmentation of Cl₃-EE2.

2. Influence of external variables on degradation

Table S1. Complete description of the experimental conditions for Set 1.

Experiment	Pharmaceutical concentrations			[NaOCl] /mg/L	pH	T /°C
	[PAR] /mg/L	[CBZ] /mg/L	[EE2] /mg/L			
1	2.000	0.5002	1.994	1.000	5	20
1b	2.000	0.5002	1.994	1.000	5	20
2	2.000	0.5002	1.994	3.000	5	20
2b	2.000	0.5002	1.994	3.000	5	20
3	2.000	0.5002	1.994	1.000	9	20
3b	2.000	0.5002	1.994	1.000	9	20
4	2.000	0.5002	1.994	3.000	9	20
4b	2.000	0.5002	1.994	3.000	9	20
5	2.000	0.5002	1.994	1.000	7	15
5b	2.000	0.5002	1.994	1.000	7	15
6	2.000	0.5002	1.994	3.000	7	15
6b	2.000	0.5002	1.994	3.000	7	15
7	2.000	0.5002	1.994	1.000	7	25
7b	2.000	0.5002	1.994	1.000	7	25
8	2.000	0.5002	1.994	3.000	7	25
8b	2.000	0.5002	1.994	3.000	7	25
9	2.000	0.5002	1.994	2.000	5	15
9b	2.000	0.5002	1.994	2.000	5	15
10	2.000	0.5002	1.994	2.000	9	15
10b	2.000	0.5002	1.994	2.000	9	15
11	2.000	0.5002	1.994	2.000	5	25
11b	2.000	0.5002	1.994	2.000	5	25
12	2.000	0.5002	1.994	2.000	9	25
12b	2.000	0.5002	1.994	2.000	9	25
Central a	2.000	0.5002	1.994	2.000	7	20
Central b	2.000	0.5002	1.994	2.000	7	20
Central c	2.000	0.5002	1.994	2.000	7	20

Table S2. Complete description of the experimental conditions for Set 2.

Experiment	Pharmaceutical concentration			[NaOCl] /mg/L	[DOM] /mg/L	pH	T /°C
	[PAR] /mg/L	[CBZ] /mg/L	[EE2] /mg/L				
1	2.000	0.5002	1.994	1.000	0.1000	7	20
1b	2.000	0.5002	1.994	1.000	0.1000	7	20
2	2.000	0.5002	1.994	3.000	0.1000	7	20
2b	2.000	0.5002	1.994	3.000	0.1000	7	20

3	2.000	0.5002	1.994	1.000	5.000	7	20
3b	2.000	0.5002	1.994	1.000	5.000	7	20
4	2.000	0.5002	1.994	3.000	5.000	7	20
4b	2.000	0.5002	1.994	3.000	5.000	7	20
5	2.000	0.5002	1.994	1.000	2.550	5	20
5b	2.000	0.5002	1.994	1.000	2.550	5	20
6	2.000	0.5002	1.994	3.000	2.550	5	20
6b	2.000	0.5002	1.994	3.000	2.550	5	20
7	2.000	0.5002	1.994	1.000	2.550	9	20
7b	2.000	0.5002	1.994	1.000	2.550	9	20
8	2.000	0.5002	1.994	3.000	2.550	9	20
8b	2.000	0.5002	1.994	3.000	2.550	9	20
9	2.000	0.5002	1.994	1.000	2.550	7	15
9b	2.000	0.5002	1.994	1.000	2.550	7	15
10	2.000	0.5002	1.994	3.000	2.550	7	15
10b	2.000	0.5002	1.994	3.000	2.550	7	15
11	2.000	0.5002	1.994	1.000	2.550	7	25
11b	2.000	0.5002	1.994	1.000	2.550	7	25
12	2.000	0.5002	1.994	3.000	2.550	7	25
12b	2.000	0.5002	1.994	3.000	2.550	7	25
13	2.000	0.5002	1.994	2.000	0.1000	5	20
13b	2.000	0.5002	1.994	2.000	0.1000	5	20
14	2.000	0.5002	1.994	2.000	5.000	5	20
14b	2.000	0.5002	1.994	2.000	5.000	5	20
15	2.000	0.5002	1.994	2.000	0.1000	9	20
15b	2.000	0.5002	1.994	2.000	0.1000	9	20
16	2.000	0.5002	1.994	2.000	5.000	9	20
16b	2.000	0.5002	1.994	2.000	5.000	9	20
17	2.000	0.5002	1.994	2.000	0.1000	7	15
17b	2.000	0.5002	1.994	2.000	0.1000	7	15
18	2.000	0.5002	1.994	2.000	5.000	7	15
18b	2.000	0.5002	1.994	2.000	5.000	7	15
19	2.000	0.5002	1.994	2.000	0.1000	7	25
19b	2.000	0.5002	1.994	2.000	0.1000	7	25
20	2.000	0.5002	1.994	2.000	5.000	7	25
20b	2.000	0.5002	1.994	2.000	5.000	7	25
21	2.000	0.5002	1.994	2.000	2.550	5	15
21b	2.000	0.5002	1.994	2.000	2.550	5	15
22	2.000	0.5002	1.994	2.000	2.550	9	15
22b	2.000	0.5002	1.994	2.000	2.550	9	15
23	2.000	0.5002	1.994	2.000	2.550	5	25
23b	2.000	0.5002	1.994	2.000	2.550	5	25
24	2.000	0.5002	1.994	2.000	2.550	9	25
24b	2.000	0.5002	1.994	2.000	2.550	9	25
Central a	2.000	0.5002	1.994	2.000	2.550	7	20
Central b	2.000	0.5002	1.994	2.000	2.550	7	20

Central c	2.000	0.5002	1.994	2.000	2.550	7	20
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Tables S1-S2 represent the experimental conditions applied for each study (Set 1 and Set 2) with each pharmaceutical. Tables S3-S8 present the results for the analysis of variance (ANOVA) for each model and each compound, along with their corresponding quadratic terms and interactions. The first step, is to ascertain the validity of both sets. Subsequently, for Set 1 with PAR, we will have:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the theoretical F -value (obtainable from the distribution tables of statistical literature) is 1.833;
- As presented in Table S3, the experimental F -value of the model for the degradation of PAR is 161.760, and therefore, significantly higher than the theoretical value above;
- The P -value for the model is 0, as indicated in Table S3, and therefore lower than the significance level ($\alpha = 0.05$);

Since the experimental P -value is lower than the significance level and the experimental F -value is higher than the critical one, there is cause and justification for the null hypothesis (H_0) of inferential statistics (that states that there is no relation between the results and the studied variables, and that the differences found between them are not statistically significant) to be safely rejected. Subsequently, there is reason therefore to safely accept the alternative hypothesis (H_a), which states the opposite. Therefore, we can conclude that the model is valid and presents statistical significance. Regarding the results, the pH (β -coefficient = 11.904 ± 0.378) and NaOCl concentration (β -coefficient = 6.384 ± 0.755) proved to be the most prominent variables. Fittingly, these variables also presented the highest F -values (71.488 for NaOCl concentration, and 994.387 for pH). The P -values for all variables were 0, thus falling below the significance level of 0.05.

For Set 2 with PAR:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the theoretical F -value (obtainable from the distribution tables of statistical literature) is 1.761;
- As presented in Table S4, the experimental F -value of the model is 52.545, and so, much higher than the theoretical value;

- The P -value for the model was also 0, and therefore lower than the significance level considered ($\alpha = 0.05$);

Just as with Set 1 of experiments, we can consider Set 2 statistically validated. As far as the results are concerned, and just as in the first model, pH (β -coefficient = 6.271 ± 0.306 ; and F -value = 419.600) and NaOCl concentration (β -coefficient = 4.566 ± 0.612 ; and F -value = 55.614) displayed the most significant influence in the degradation of PAR. It is also crucial to highlight the role of DOM, that, despite not proving to be more influential than pH and NaOCl levels, was also deemed significant (β -coefficient = -1.498 ± 0.250). More importantly, its negative signal means that degradation of PAR decreased with the increase in DOM levels, which is expectable, since DOM is known to actively compete with other organic compounds for the available chlorine in solution.

In the case of CBZ, and Set 1, we have:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the theoretical F -value (obtainable from the distribution tables of statistical literature) is 1.833;
- The experimental F -value of the model is 15.016, as displayed in [Table S5](#), and therefore, significantly higher than the theoretical value above;
- The P -value for the model is 0, just as the previous cases, and therefore lower than the significance level of 5%;

Thus, we can consider the model statistically valid. The results, however, proved once again that NaOCl levels (β -coefficient = 2.431 ± 0.506 ; and F -value = 23.116) and pH (β -coefficient = -2.199 ± 0.253 ; and F -value = 75.663) were the most influential variables on degradation.

For Set 2 with CBZ, we have:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the theoretical F -value (obtainable from the distribution tables of statistical literature) is 1.761;
- The experimental F -value of the model is 3.495, as shown in [Table S6](#), and so, higher than the theoretical value just presented above;
- The P -value for the model was 0.0012, which means it also falls below the significance level considered ($\alpha = 0.05$);

Again, this allows us to conclude that the model is also statistically valid. Results show, yet again, that pH (β -coefficient = -1.289 ± 0.302 ; and F -value = 18.248) and NaOCl (β -coefficient = 0.916 ± 0.603 ; and F -value = 2.305) levels were the most influential in the degradation of the compound.

Finally, regarding Set 1 with EE2:

- For a significance level of 5% ($\alpha = 0.05$) and 9 degrees of freedom, the theoretical F -value (obtainable from the distribution tables of statistical literature) is 1.833;
- The experimental F -value of the model, presented in Table S7, is 178.315, which is quite higher than the theoretical value;
- The P -value of this model is also 0, and therefore lower than the significance level of 5%;

The model is thus considered statistically valid. As for the experimental results, the temperature showed no influence whatsoever in the degradation of the compound, whereas the pH (β -coefficient = 14.479 ± 0.415 ; and F -value = $1.215\text{E}+03$) and NaOCl (β -coefficient = 12.049 ± 0.831 ; and F -value = 210.329) displayed quite a significant impact.

As for Set 2 with EE2:

- For a significance level of 5% ($\alpha = 0.05$) and 14 degrees of freedom, the theoretical F -value (obtainable from the distribution tables of statistical literature) is 1.761;
- The experimental F -value of the model, presented in Table S8, is 28.851, and so, much higher than the critical value;
- The P -value for the model was 0, which means it is lower than the significance level considered ($\alpha = 0.05$);

Once again, the model is considered statistically validated. Results show that NaOCl concentration played the most significant impact on the degradation of the compound (β -coefficient = 10.178 ± 0.740 ; and F -value = 189.225), followed by the pH (β -coefficient = 3.621 ± 0.370 ; and F -value = 95.783). DOM deserves also a mention, despite falling behind the previous variables in significance (β -coefficient = -1.358 ± 0.302 ; and F -value = 20.215), but its effect was indeed meaningful in the sense that its increase led to a decrease in the degradation rate of EE2, as was expected.

Overall tendencies of these studies are presented in a more intuitive manner in the form of response surfaces, displayed in [Figures S16-S21](#). The graphical representations convey the degradation rates as a function of the most influential variables for each model and pharmaceutical.

Table S3. ANOVA for Set 1 of experiments with PAR.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1.328E+04	9	1.475E+03	161.760	0		
	Error	155.055	17	9.121				
	Adjusted total	1.343E+04	26	516.676				
Factors	Intercept	682.823	1	682.823	74.863	0	15.087	1.744
	[NaOCl] /mg/L (A)	652.036	1	652.036	71.488	0	6.384	0.755
	pH (B)	9.070E+03	1	9.070E+03	994.387	0	11.904	0.378
	T /°C (C)	303.456	1	303.456	33.270	0	0.871	0.151
	AB	599.445	1	599.445	65.722	0	5.327	0.657
	AC	6.302E-02	1	6.302E-02	6.909E-03	0.9347	-5.462E-02	0.657
	BC	177.190	1	177.190	19.427	0.0004	2.896	0.657
	AA	0.309	1	0.309	3.389E-02	0.8561	-0.144	0.782
	BB	2.221E+03	1	2.221E+03	243.548	0	12.205	0.782
	CC	32.352	1	32.352	3.547	0.0769	1.473	0.782
Model check	Main	1.003E+04	3	3.342E+03				
	Int	776.699	3	258.900	28.385	0		
	Int + Sqr	2.477E+03	3	825.545	90.511	0		
	Squ	2.477E+03	3	825.546	90.511	0		
	Error	155.055	17	9.121				
Lack of fit	Lack of fit	154.283	3	51.428	932.489	0		
	Pure error	0.772	14	5.515E-02				
	Total error	155.055	17	9.121				

Table S4. ANOVA for Set 2 of experiments with PAR.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	6.619E+03	14	472.799	52.545	0		
	Error	323.926	36	8.998				
	Adjusted total	6.943E+03	50	138.862				
Factors	Intercept	747.657	1	474.657	83.092	0	15.787	1.732
	[NaOCl] /mg/L (A)	500.415	1	500.415	55.614	0	4.566	0.612
	[DOM] /mg/L (B)	323.401	1	323.401	35.942	0	-1.498	0.250
	pH (C)	3.776E+03	1	3.776E+03	419.600	0	6.271	0.306
	T /°C (D)	24.462	1	24.462	2.719	0.1079	0.202	0.122
	AB	0.451	1	0.451	5.015E-02	0.8241	-0.114	0.509
	AC	605.172	1	605.172	67.257	0	4.175	0.509
	AD	41.178	1	41.178	4.576	0.0393	1.089	0.509
	BC	176.438	1	176.438	19.609	0.0001	-2.254	0.509
	BD	8.262	1	8.262	0.918	0.3443	-0.488	0.509
	CD	62.552	1	62.552	6.952	0.0123	1.342	0.509
	AA	13.907	1	13.907	1.546	0.2218	0.659	0.530
	BB	22.756	1	22.756	2.529	0.1205	0.843	0.530
	CC	679.385	1	679.385	75.504	0	4.604	0.530
	DD	5.624	1	5.624	0.625	0.4343	-0.419	0.530
Model check	Main	4.624E+03	4	1.156E+03				
	Int	894.053	6	149.009	16.560	0		
	Int + Sqr	1.101E+03	4	275.328	30.599	0		
	Squ	1.101E+03	4	275.328	30.599	0		
	Error	323.926	36	8.998				
Lack of fit	Lack of fit	321.627	10	32.163	363.758	0		
	Pure error	2.299	26	8.842E-02				
	Total error	323.926	36	8.998				

Table S5. ANOVA for Set 1 of experiments with CBZ.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	552.656	9	61.406	15.016	0		
	Error	69.518	17	4.089				
	Adjusted total	622.173	26	23.930				
Factors	Intercept	849.074	1	849.074	207.634	0	16.823	1.168
	[NaOCl] /mg/L (A)	94.527	1	94.527	23.116	0.0002	2.431	0.506
	pH (B)	309.408	1	309.408	75.663	0	-2.199	0.253
	T /°C (C)	31.500	1	31.500	7.703	0.0130	-0.281	0.101
	AB	20.161	1	20.161	4.930	0.0403	-0.977	0.440
	AC	14.178	1	14.178	3.467	0.0800	-0.819	0.440
	BC	57.459	1	57.459	14.051	0.0016	1.649	0.440
	AA	17.938	1	17.938	4.387	0.0515	-1.097	0.524
	BB	0.432	1	0.432	0.106	0.7492	0.170	0.524
	CC	0.871	1	0.871	0.213	0.6503	0.242	0.524
Model check	Main	435.435	3	145.145				
	Int	91.798	3	30.599	7.483	0.0021		
	Int + Sqr	25.422	3	8.474	2.072	0.1419		
	Squ	25.422	3	8.474	2.072	0.1419		
	Error	69.518	17	4.089				
Lack of fit	Lack of fit	68.073	3	22.691	219.902	0		
	Pure error	1.445	14	0.103				
	Total error	69.518	17	4.089				

Table S6. ANOVA for Set 2 of experiments with CBZ.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	427.684	14	30.549	3.495	0.0012		
	Error	314.657	36	8.740				
	Adjusted total	742.342	50	14.847				
Factors	Intercept	1.187E+03	1	1.187E+03	135.832	0	19.893	1.707
	[NaOCl] /mg/L (A)	20.148	1	20.148	2.305	0.1377	0.916	0.603
	[DOM] /mg/L (B)	6.795	1	6.795	0.777	0.3838	-0.217	0.246
	pH (C)	159.496	1	159.496	18.248	0.0001	-1.289	0.302
	T /°C (D)	4.293	1	4.293	0.491	0.4879	-8.458E-02	0.121
	AB	0.627	1	0.627	7.176E-02	0.7903	0.134	0.502
	AC	56.711	1	56.711	6.488	0.0153	-1.278	0.502
	AD	14.285	1	14.285	1.634	0.2093	-0.641	0.502
	BC	10.998	1	10.998	1.258	0.2694	0.563	0.502
	BD	7.547	1	7.547	0.863	0.3590	-0.466	0.502
	CD	6.213	1	6.213	0.711	0.4047	0.423	0.502
	AA	22.358	1	22.358	2.558	0.1185	-0.835	0.522
	BB	0.395	1	0.395	4.518E-02	0.8329	-0.111	0.522
	CC	29.317	1	29.317	3.354	0.0753	0.956	0.522
	DD	16.012	1	16.012	1.832	0.1843	-0.707	0.522
Model check	Main	190.731	4	47.683				
	Int	96.381	6	16.063	1.838	0.1192		
	Int + Sqr	140.572	4	35.143	4.021	0.0085		
	Sqr	140.573	4	35.143	4.021	0.0085		
	Error	314.657	36	8.740				
Lack of fit	Lack of fit	312.001	10	31.200	305.398	0		
	Pure error	2.656	26	0.102				
	Total error	314.657	36	8.740				

Table S7. ANOVA for Set 1 of experiments with EE2.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	1.772E+04	9	1.969E+03	178.315	0		
	Error	187.758	17	11.045				
	Adjusted total	1.791E+04	26	688.943				
Factors	Intercept	6.821E+03	1	6.821E+03	617.596	0	47.683	1.919
	[NaOCl] /mg/L (A)	2.323E+03	1	2.323E+03	210.329	0	12.049	0.831
	pH (B)	1.342E+04	1	1.342E+04	1.215E+03	0	14.479	0.415
	T /°C (C)	4.895	1	4.895	0.443	0.5145	0.111	0.166
	AB	340.083	1	340.083	30.792	0	4.012	0.723
	AC	38.676	1	38.676	3.502	0.0786	-1.353	0.723
	BC	102.961	1	102.961	9.322	0.0072	2.208	0.723
	AA	40.717	1	40.717	3.687	0.0718	1.652	0.861
	BB	889.573	1	889.573	80.544	0	-7.724	0.861
	CC	149.932	1	149.932	13.575	0.0018	3.171	0.861
Model check	Main	1.575E+04	3	5.249E+03				
	Int	481.720	3	160.573	14.539	0.0001		
	Int + Sqr	1.497E+03	3	499.135	45.193	0		
	Squ	1.497E+03	3	499.135	45.193	0		
	Error	187.758	17	11.045				
Lack of fit	Lack of fit	39.206	3	13.069	1.232	0.3352		
	Pure error	148.553	14	10.611				
	Total error	187.758	17	11.045				

Table S8. ANOVA for Set 2 of experiments with EE2.

		Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	F-value	P-value	β -coefficients	Standard error of β
Summary	Model	5.307E+03	14	379.062	28.851	0		
	Error	472.992	36	13.139				
	Adjusted total	5.780E+03	50	115.597				
Factors	Intercept	5.721E+03	1	5.721E+03	435.448	0	43.670	2.093
	[NaOCl] /mg/L (A)	2.486E+03	1	2.486E+03	189.225	0	10.178	0.740
	[DOM] /mg/L (B)	265.601	1	265.601	20.215	0.0001	-1.358	0.302
	pH (C)	1.258E+03	1	1.258E+03	95.783	0	3.621	0.370
	T /°C (D)	118.815	1	118.815	9.043	0.0048	0.445	0.148
	AB	8.674	1	8.674	0.660	0.4218	-0.500	0.615
	AC	33.661	1	33.661	2.562	0.1182	0.985	0.615
	AD	516.650	1	516.650	39.323	0	3.857	0.615
	BC	296.826	1	296.826	22.592	0	2.924	0.615
	BD	71.163	1	71.163	5.416	0.0257	1.432	0.615
	CD	179.077	1	179.077	13.630	0.0007	2.271	0.615
	AA	11.066	1	11.066	0.842	0.3649	0.588	0.640
	BB	14.426	1	14.426	1.098	0.3017	-0.671	0.640
	CC	10.145	1	10.145	0.772	0.3854	-0.563	0.640
	DD	4.198	1	4.198	0.320	0.5754	-0.362	0.640
Model check	Main	4.129E+03	4	1.032E+03				
	Int	1.106E+03	6	184.342	14.030	0		
	Int + Sqr	71.789	4	17.947	1.366	0.2650		
	Squ	71.789	4	17.947	1.366	0.2650		
	Error	472.992	36	13.139				
Lack of fit	Lack of fit	469.437	10	46.944	343.373	0		
	Pure error	3.555	26	0.137				
	Total error	472.992	36	13.139				

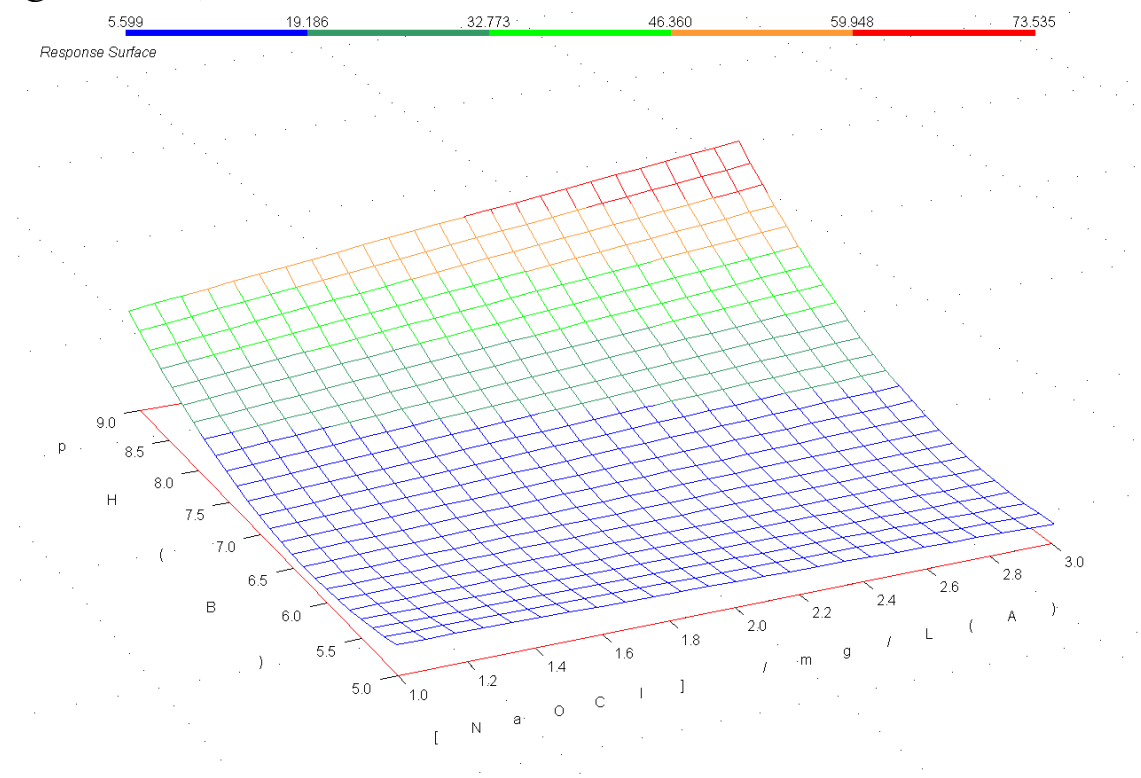


Figure S17. Response surface obtained for Set 1 of experiments with PAR.

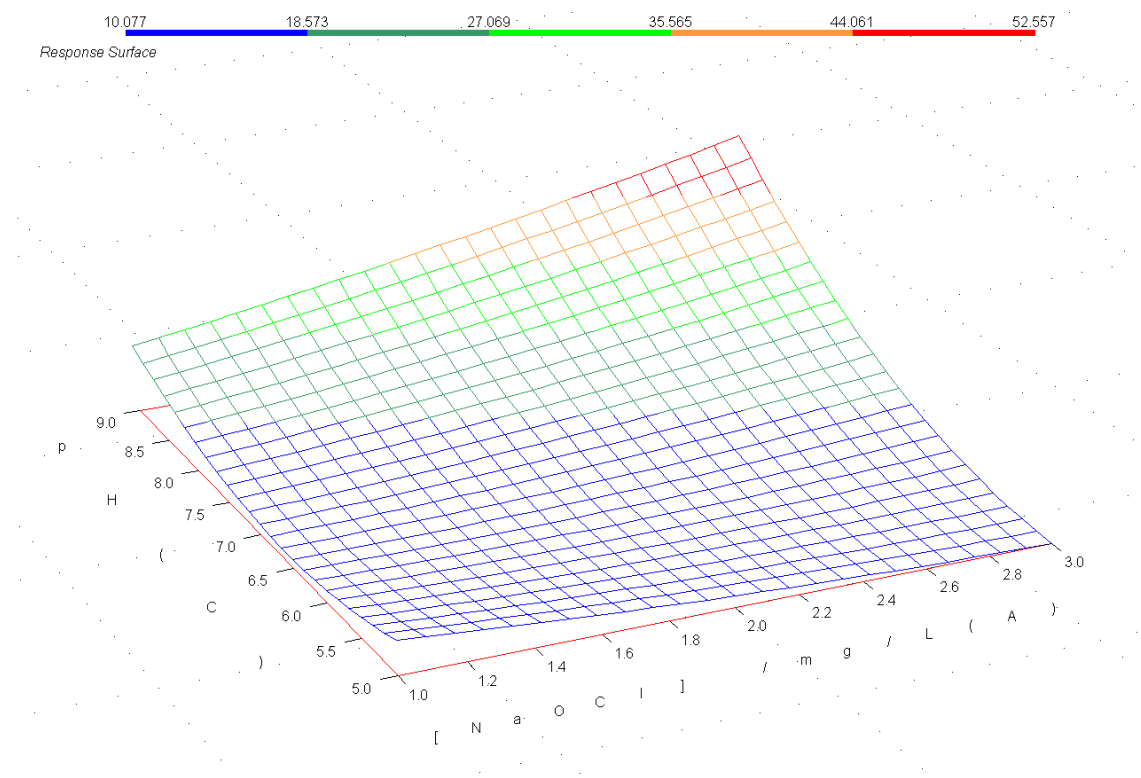


Figure S18. Response surface obtained for Set 2 of experiments with PAR.

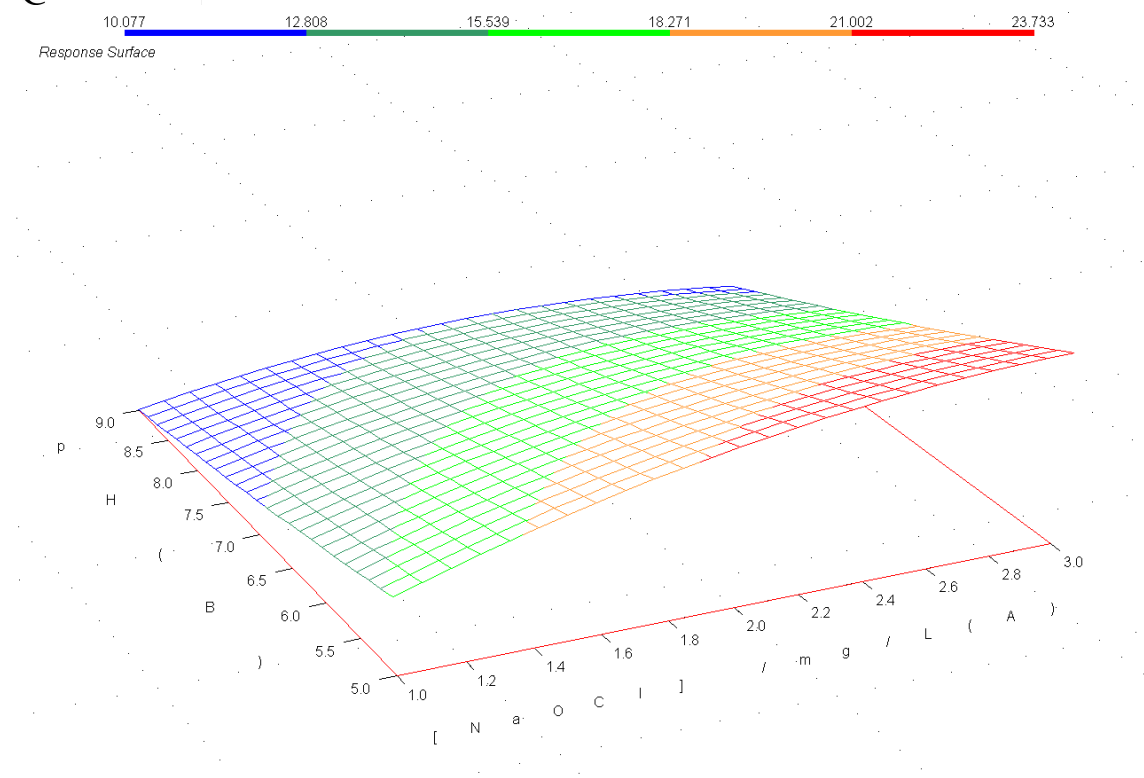


Figure S19. Response surface obtained for Set 1 of experiments with CBZ.

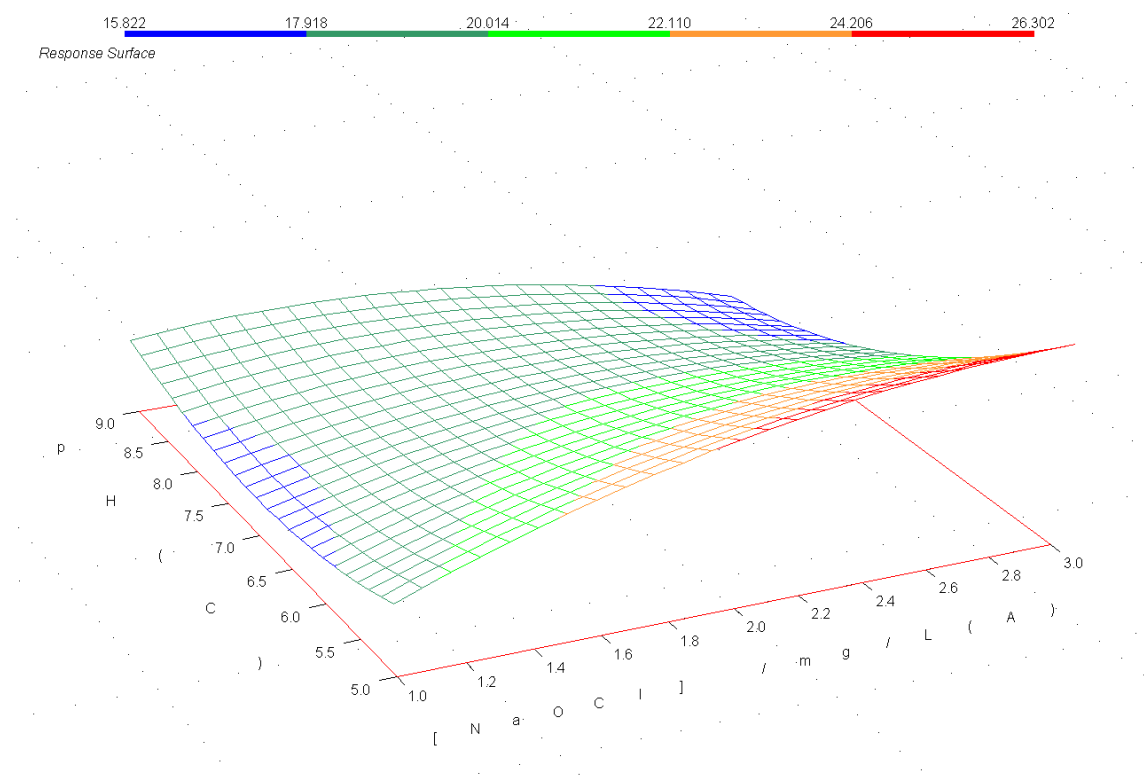


Figure S20. Response surface obtained for Set 2 of experiments with CBZ.

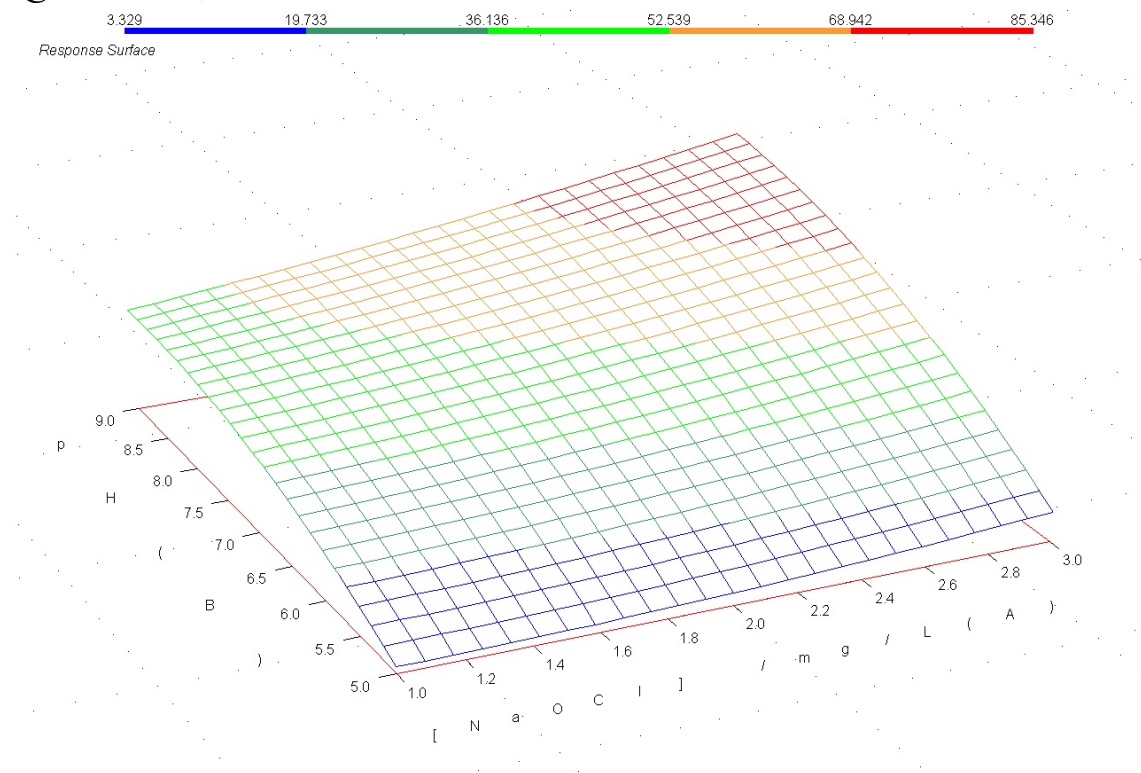


Figure S21. Response surface obtained for Set 1 of experiments with EE2.

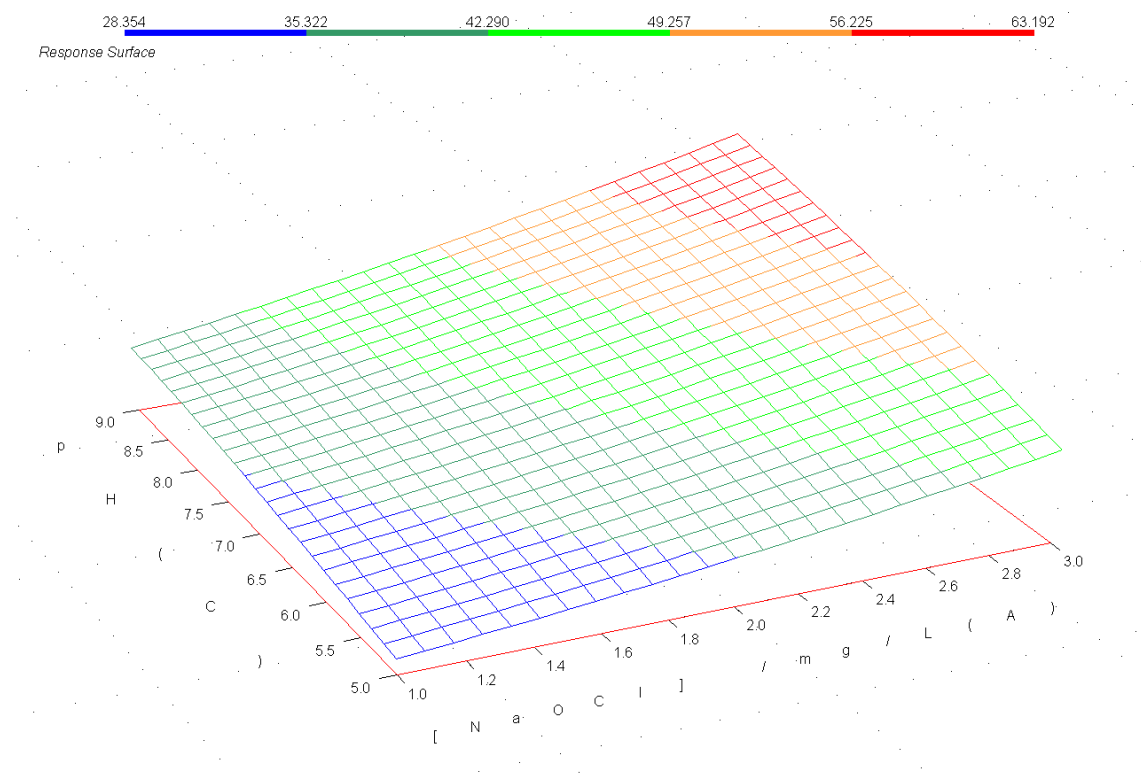


Figure S22. Response surface obtained for Set 2 of experiments with EE2.

Chapter 4 – Computational determination of physical-chemical properties, environmental persistence and long-range transport potential of selected UV filters, pharmaceuticals and respective disinfection by-products

4.1. Introduction

Physical-chemical properties are of paramount importance in the understanding of the fate and behaviour of emerging pollutants (EPs) within the different environmental compartments, because their degradation profiles, persistence, ecotoxicity and ability to influence normal biological mechanisms, are directly dependent on these properties, and therefore, on their molecular structure [1]. Figure 1 exposes the connection between these attributes, fate and behaviour of chemicals in the environment and their toxicological effects.

The evaluation of environmental fate of pollutants and their eco-toxicological profiles, are almost entirely determined by factors that are influenced by their inherent physical-chemical properties. For example, dispersion of pollutants in and between the different environmental compartments (atmosphere, natural water courses, soils and sediments), determines the rate and significance of exposure of humans, animals or the ecosystems to those compounds. This mobility of the compounds, is very much influenced by prominent physical-chemical attributes such as vapor pressure, volatility and water solubility [2-5]. Many organic pollutants, in fact, display a very significant long-range transport (LRT) potential, which means they are able to travel through thousands of kilometres from their point of inclusion into the compartment and lead to widespread contamination [6].

Since the distinct chemical reactivity of EPs is defined by their characteristic physical-chemical properties, these can be used to predict their potential environmental fate and behaviour, and ultimately, their general toxicity (towards animals, humans and the ecosystems) [1]. In more detail, from the intrinsic, structural

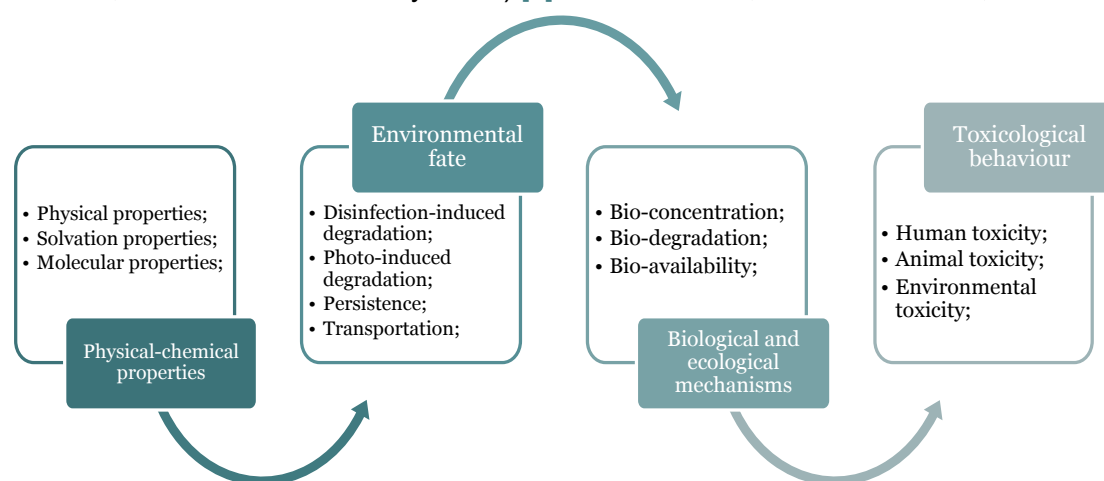


Figure 1. Relationship between physical-chemical properties, environmental fate and behavior, eco-biological mechanisms and eco-toxicological effects. Adapted from [1].

characteristics of the compounds, such as molecular mass, typical functional groups and chemical bonds displayed, the properties can be estimated or determined, such as water solubility, vapor pressure, partitioning coefficients, etc. From these attributes, the transformation and distribution in the environment can be predicted, such as persistence, transportation potential, bio-accumulation, bio-concentration, etc. Ultimately, this data will allow the prediction of the eco-toxicological profiles of the compounds [1, 7]. A more thorough analysis of these physical-chemical factors discriminated in Figure 1, will follow [1]:

- Physical properties: this set of attributes regards the specific physical and structural state of the compound, and include the molecular mass, freezing temperature, boiling temperature, melting temperature, infrared spectrum, electronic characteristics, viscosity and density;
- Solvation properties: this set of factors determines the way through which the compounds interact with different phases, or more specifically, the way it partitions into those; the attributes are divided into three sub-groups:
 - Phase-partitioning: partition coefficients represent the ratio between the concentrations of a certain compound in two different, immiscible phases at equilibrium, more prominently, the octanol-water partition coefficient ($\text{Log } K_{ow}$), but also the coefficients from environmental partitioning, such as air-water ($\text{Log } K_{aw}$ or Henry's law) and octanol-air ($\text{Log } K_{oa}$);
 - Solubility: determines the ability of the compound to be dissolved into a solvent, more importantly in environmental terms, water;
 - Colligative properties: these attributes are generally not very important, but they represent properties that are not dependent on the chemical species but rather the ratio between the quantity of compound particles and the quantity of solvent molecules in a given solution;
- Molecular properties: this group describes attributes that are a function of molecular structure, shape and size, for instance, electronic properties such as polarizability;

It is also possible to compile the most prominent properties of each type mentioned above and connect them with their environmentally-relevant interactions. This association is presented in Figure 2.

Conventionally, the use of physical-chemical properties in the evaluation of a given compound, starts with the identification and classification of the most likely physical impacts of said chemical, from the manufacturing stage itself, to the handling, transportation, consumer applications and end of lyfe-cycle phase [1]. Next, comes the assessment of the most probable environmental compartments into which the compounds may partition (atmosphere, water, soils and sediments). This evaluation is carried out on the basis of the analysis of properties such as melting point, boiling point and vapour pressure. Partitioning into the atmosphere is a strong concern, for highly volatile chemicals, for example. On the other hand, compounds with tendency towards association with organic carbon or lipophilic phases, will concentrate in soils, sediments and also the food chain. Increased water solubility, will indicate strong probability towards dissolution in the water phase, and the Log K_{ow} may help to enlighten the fate and behaviour of the chemical in the water-sediment context. The higher the Log K_{ow} , the higher the probability of the compound being absorbed by the food-chain. Transformation or degradation processes in the environment must also be highlighted, since by-products might display different physical-chemical properties and therefore, distinct fate or behaviour within the environmental compartments [1]. Biological and ecological mechanisms are also an important factor to account. Organisms in general will absorb, metabolize and eliminate chemicals at different rates. If the metabolization and excretion processes are slow, the compounds will accumulate in the organisms and lead to more significant and more prolonged exposure [1, 8].

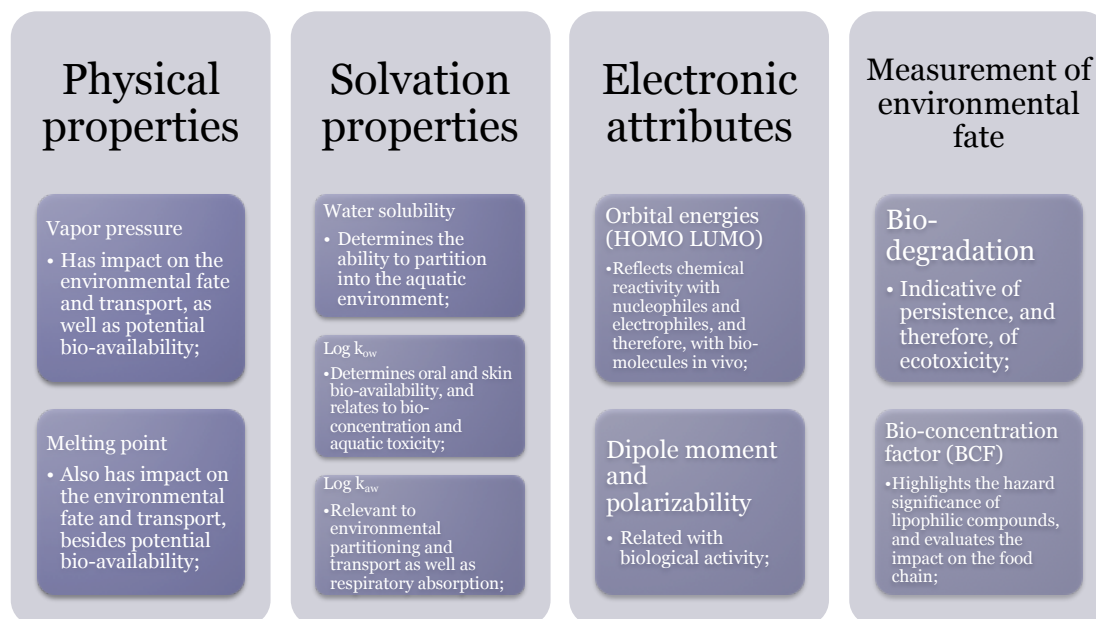


Figure 2. Some of the most environmentally-relevant physical-chemical properties and their expected effects in the ecosystems.

Persistence of an organic pollutant in a given environmental compartment, represents the amount of time in which the chemical will remain in that compartment, before it is bio-chemically transformed or partitioned into another compartment [1, 9]. The more significant the persistence of a pollutant, the more will the concentration increase, resulting in more prominent bio-accumulation and more concerning environmental impact. An atmospheric oxidation half-life period higher than 2 days, has been defined as a strong physical-chemical property indicator of atmospheric persistence. Additionally, compounds with $\text{Log } K_{ow}$ between -5 and -1, have been labelled as susceptible to long-range transportation [1, 10]. Persistence is also a function of bio-degradation mechanisms, which will depend on the environmental conditions, micro-organisms present and structural properties of the compounds. These processes may yield the formation of by-products with distinct, and possibly more concerning, physical-chemical properties, fate, behaviour and toxicological profiles. In similar fashion, persistence will also depend on chemical degradation processes, such as photo-induced or disinfection-induced mechanisms. The type of environmental compartment in question, as well as other conditions such as the pH and even the concentration of relevant species, like active chlorine, will influence this

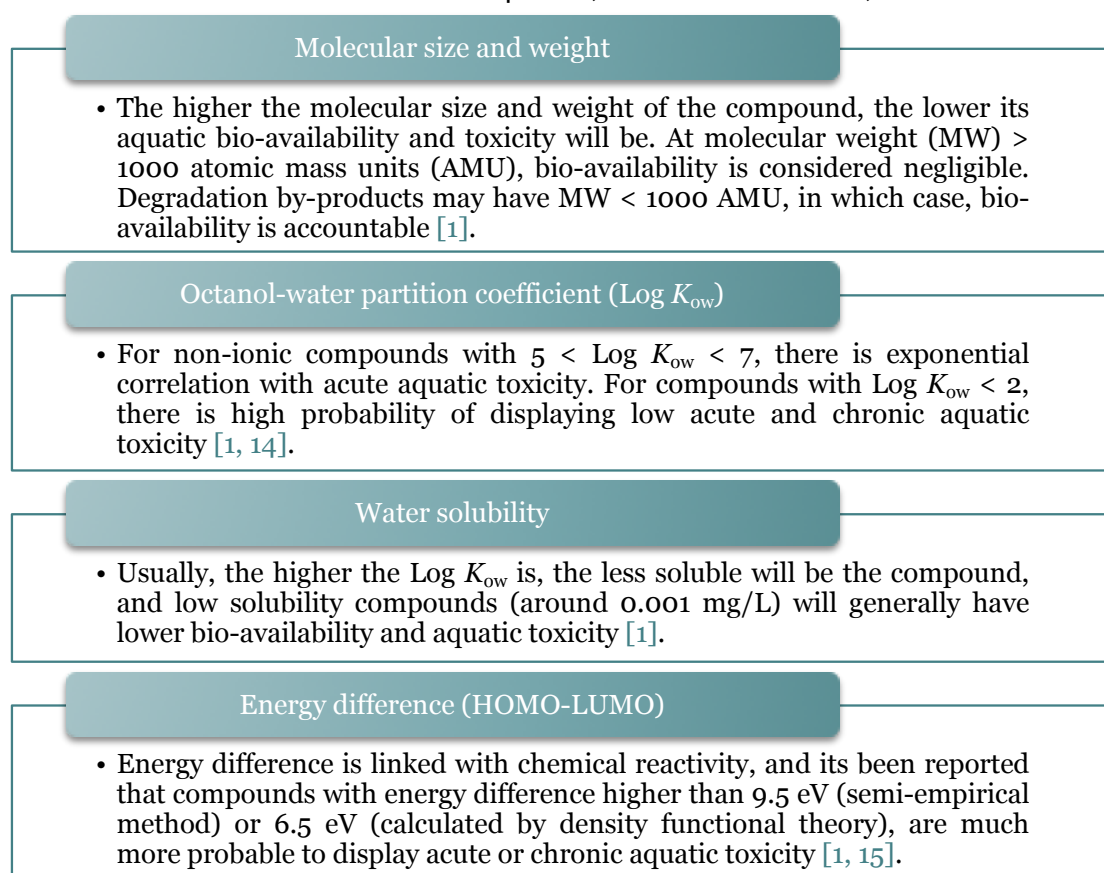


Figure 3. Prominent properties in regards to aquatic bio-availability and toxicity, and the way they are associated with those environmental aspects. Inspired by and adapted from [1].

chemically-induced degradation. Formation of troublesome by-products, is also a concerning issue in this case [1, 11].

Bio-accumulation of organic pollutants, is more commonly known as and represented by the bio-concentration factor (BCF), and it regards the ratio between the amount of pollutant in an aquatic organism and the amount of compound in the water course, under conditions of equilibrium [1]. Alternatively, the Log K_{ow} also presents an adequate representation of the proclivity on which chemicals will be distributed between the lipid phase of aquatic organisms or the water phase. Highly lipophilic compounds, with Log $K_{ow} > 5$, have been determined to display the most significant tendency to bio-accumulate. This process also influences bio-availability, that is, the process of an organism absorbing a chemical through its usual diet. In cases of Log $K_{ow} > 5$, chemicals will be essentially absorbed through diet, whereas absorption through the water will occur when Log $K_{ow} < 5$ [1, 12]. As for the prediction of bio-accumulation potential of organic pollutants, compounds with no significant bio-accumulation will usually display BCF below 1000, bio-accumulative compounds will have BCF between 1000 and 5000, whereas highly bio-accumulative chemicals will typically display a BCF higher than 5000 [1, 13].

Bio-availability and toxicity are two important environmental factors than can also be predicted and evaluated according to the physical-chemical attributes of organic pollutants. Bio-availability refers to the process through which pollutants are absorbed from the environmental compartment by living organisms, ultimately entering their circulatory system. From that point, all cells and tissues of the organism will be directly exposed to the contaminant [1]. Bio-availability is directly linked to toxicity, since for a given organic pollutant to display a potential toxic behaviour, said chemical must first be bio-available at levels that will allow the compound or its degradation by-products to reach biochemical targets and induce a toxicological effect. This means that, if the bio-availability of an organic pollutant can be reduced or even blocked, its toxicity can also be reduced [1, 14]. In the aquatic environmental compartments, bio-availability is positively correlated with Log K_{ow} and also linked with other properties such as water solubility, molecular size and ionization state. This exemplifies how reactivity attributes can be used to predict and evaluate chemical reactivity of organic pollutants and how it can then be associated with biological activity and impact [1]. Figure 3 highlights how important properties such as molecular size, weight, Log K_{ow} and water solubility, seem to correlate with aquatic toxicity.

4.2. Methods

The computational calculation of the most environmentally-relevant physical-chemical properties, was carried out in two stages. First, all of the physical-chemical parameters were determined by means of the EPI Suite™ [16]. This important computational tool, developed by the United States Environmental Protection Agency (USEPA) and the Syracuse Research Corporation, determines physical-chemical properties based on structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs). SAR and QSAR models are theoretical representations that allow the quantitative or qualitative prediction of physical-chemical, biological and environmental fate properties of a compound, from the analysis of its molecular structure. The chemicals studied were those approached in sections 2 and 3 of this work, that is to say, the UV filters IMC, DHHB and BP8, along with all their identified or detected degradation by-products, as well as the pharmaceuticals PAR, CBZ, EE2 and their respective detected by-products. The determined parameters were then used to calculate the overall persistence (P_{ov}) and long-range transport potential (LRTP) of all the compounds, with the help of the P_{ov} and LRTP risk assessment screening tool from the Organization for Economic Cooperation and Development (OECD). This tool is a fugacity-based multimedia mass balance unit-world model, that considers three bulk compartments, that is, the soil's superficial layer, the ocean's superficial layer, and the troposphere (<http://www.oecd.org/chemicalsafety/risk-assessment/oecd-pov-and-lrtp-screening-tool.htm>) [17].

4.3. Results and discussion

4.3.1. Determination of physical-chemical properties

Several factors influence and control the distribution and fate of organic pollutants in the aquatic environment. The sources of contamination are naturally relevant (agricultural applications and runoff, incineration and landfills, industrial and municipal effluents, etc), but then are included the most prominent aspects such as the physical-chemical properties of the contaminants (water solubility, polarity, volatility, acid-base character, etc), the environmental conditions [water temperature, altitude, wind, rain, water pH, presence of dissolved organic matter (DOM), the water current or flow rate, etc] and persistence or degradation aspects (photo-induced degradation, chemically-induced degradation and bio-degradation). The most important physical-chemical properties of all the studied compounds as well as their degradation by-products, have been compiled in Table 1.

Figure 4 presents the results obtained for the boiling temperature (T_b), the vapour pressure (P_v), the Henry's constant (K_H) and the water solubility of the compounds. The results have been discriminated in logarithmic scale, for better understanding and

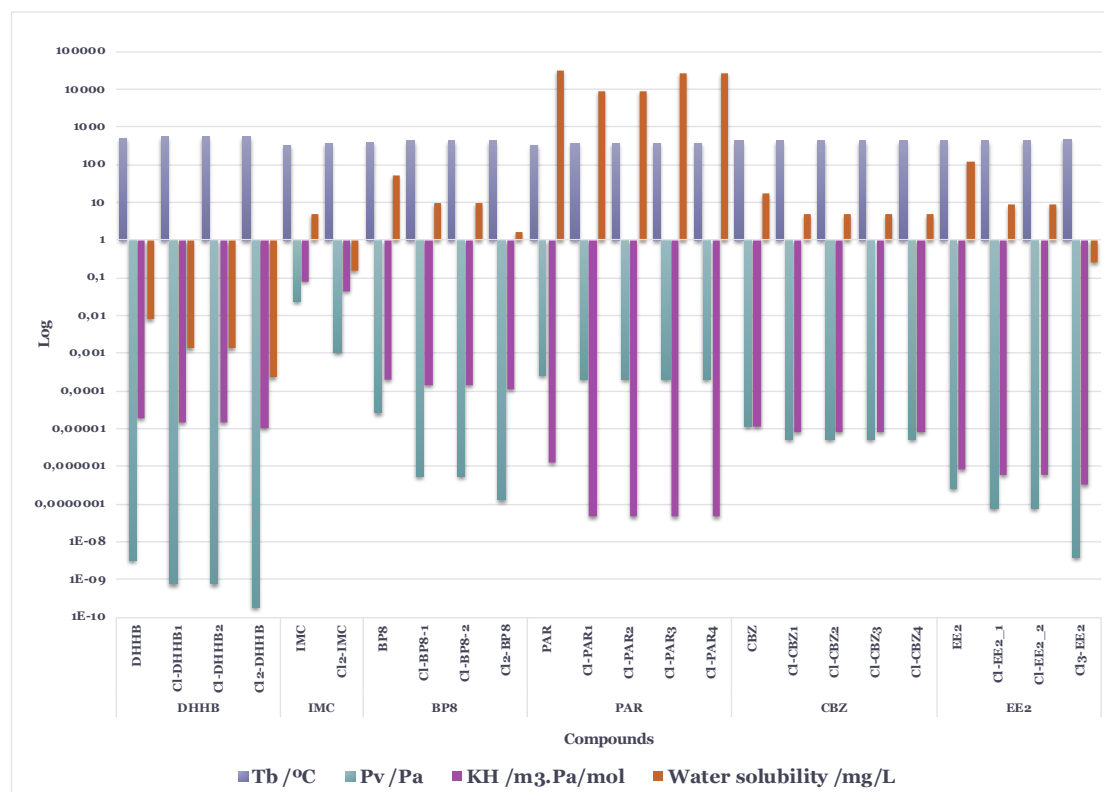


Figure 4. Graphical representation of the physical-chemical properties boiling temperature (T_b), vapour pressure (P_v), Henry's constant (K_H) and water solubility, of all the compounds and respective by-products. The vertical axis scale is logarithmic.

analogy. As far as the T_b and P_v are concerned, these properties provide valuable information regarding the proclivity of the compounds to evaporate from the water courses and enter the atmosphere.

Table 1. Physical-chemical properties determined for the studied compounds and corresponding, identified degradation by-products.

Compound	T _b /°C	P _v /Pa	Log K _{ow}	Log K _{oa}	Log K _{aw}	Log K _{oc}	K _H /m ³ .Pa/mol	Water solubility /mg/L	BCF /L/kg (wet weight)	
DHHB	DHHB	504.84	3.33E-09	6.5434	14.652	-8.112	4.7742	1.92E-05	0.008191	2506.109
	Cl-DHHB-1	523.53	7.98E-10	7.1879	15.432	-8.242	5.1338	1.42E-05	0.001407	2798.981
	Cl-DHHB-2	523.53	7.98E-10	7.1879	15.432	-8.242	5.1338	1.42E-05	0.001407	2798.981
	Cl ₂ -DHHB	542.23	1.90E-10	7.8324	16.201	-8.371	5.4878	1.05E-05	0.0002401	1352.073
IMC	IMC	324.44	0.0251	4.3288	8.837	-4.507	3.3105	7.71E-02	4.858	333.426
	Cl ₂ -IMC	363.12	0.00106	5.6178	10.387	-4.767	4.024	4.23E-02	0.155	2365.920
BP8	BP8	398.24	2.59E-05	3.8216	10.914	-7.094	3.6233	2.00E-04	52.73	40.087
	Cl-BP8-1	416.94	5.58E-07	4.4661	11.694	-7.224	3.9828	1.48E-04	9.51	106.905
	Cl-BP8-2	416.94	5.58E-07	4.4661	11.694	-7.224	3.9828	1.48E-04	9.51	106.905
	Cl ₂ -BP8	435.63	1.30E-07	5.1106	12.465	-7.355	4.3368	1.10E-04	1.689	284.446
PAR	PAR	340.65	0.000259	0.2685	9.408	-8.948	1.321	1.29E-06	3.04E+04	3.162
	Cl-PAR-1	359.47	0.000198	0.913	11.621	-10.711	1.5699	4.82E-08	8583	3.162
	Cl-PAR-2	359.47	0.000198	0.913	11.621	-10.711	1.5699	4.82E-08	8583	3.162
	Cl-PAR-3	359.47	0.000198	0.3496	11.061	-10.711	1.2601	4.82E-08	2.60E+04	3.162
	Cl-PAR-4	359.47	0.000198	0.3496	11.061	-10.711	1.2601	4.82E-08	2.60E+04	3.162
CBZ	CBZ	410.02	1.17E-05	2.2484	10.805	-8.355	2.2268	1.10E-05	17.66	19.231
	Cl-CBZ-1	428.72	5.46E-06	2.8929	11.375	-8.485	2.4702	8.12E-06	4.755	37.670
	Cl-CBZ-2	428.72	5.46E-06	2.8929	11.375	-8.485	2.4702	8.12E-06	4.755	37.670
	Cl-CBZ-3	428.72	5.46E-06	2.8929	11.375	-8.485	2.4702	8.12E-06	4.755	37.670
	Cl-CBZ-4	428.72	5.46E-06	2.8929	11.375	-8.485	2.4702	8.12E-06	4.755	37.670
EE2	EE2	411.21	2.60E-07	4.1159	13.159	-9.489	2.7105	8.04E-07	116.4	122.462
	Cl-EE2-1	429.91	7.90E-08	4.7604	14.379	-9.619	3.3134	5.96E-07	8.543	642.688
	Cl-EE2-2	429.91	7.90E-08	4.7604	14.379	-9.619	3.3134	5.96E-07	8.543	642.688
	Cl ₃ -EE2	467.30	3.75E-09	6.0494	15.929	-9.879	4.1713	3.27E-07	0.2579	4549.881

From the analysis of Table 1, as well as the analogy made in Figure 4, it can be concluded that all the compounds have slightly distinct boiling points, although in the same order of magnitude, ranging from 324.44°C, the lowest and in the case of IMC, and 542.23°C, the highest and in the case of the dichlorinated analogue of DHHB. According to the USEPA [18], semi-volatile organic compounds will display T_b between 240-400°C, whereas volatile compounds will have T_b between 100-260°C and highly-volatile organic pollutants will display T_b between 0-100°C. From this perspective, it is safe to categorize all the compounds from Table 1 as semi-volatile organic pollutants. The values for P_v and K_H also seem to confirm these indications. Compounds with higher K_H , will have more tendency to volatilize and be distributed in the atmosphere, whereas lower values for K_H will mean that the compound will be more concentrated in the water phase. From the analysis of Figure 4, all compounds show low K_H values, nonetheless, IMC and its dichlorinated by-product display the highest values of the entire set, which means they are indeed the most volatile compounds of the group. The P_v values are also, as expected, quite low for all the compounds, since they were classified earlier as semi-volatile. The least volatile compounds of the group, DHHB

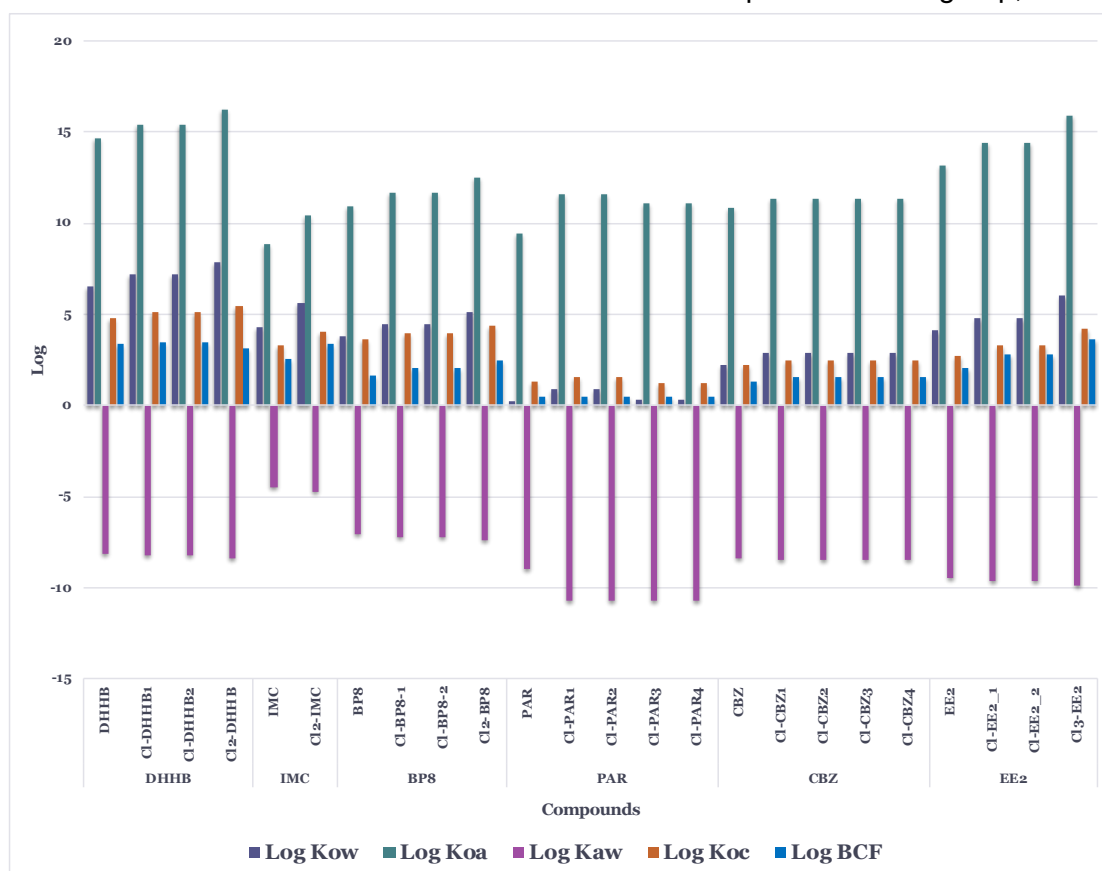


Figure 5. Graphical representation of the octanol-water ($\text{Log } K_{ow}$), octanol-air ($\text{Log } K_{oa}$), air-water ($\text{Log } K_{aw}$) and the soil organic carbon-water ($\text{Log } K_{oc}$) partition coefficients, as well as the bio-concentration factors (Log BCF). All results are present in logarithmic scale.

and its by-products, have the lowest P_v values, whereas IMC and its dichlorinated analogue, naturally display the opposite trend. Another trend verified by the analysis of the results from Table 1 or Figure 4, is that the DBPs of the studied compounds display P_v values lower than their parental compounds, which indicates that their volatility is less significant than the original chemicals. Two noteworthy exceptions to this trend, were the pharmaceuticals PAR and CBZ, which displayed very similar P_v values between the original compounds and respective by-products.

Water solubility is another important property, since it is strongly connected to bio-availability and the inherent proclivity towards absorption of pollutants by organisms. Additionally, highly hydrophilic pollutants will be subjected to transportation in the water bodies over longer distances, which means that the exposure range and significance will also increase. The higher the water solubility of an organic pollutant, the less likely it will be to volatilize and enter the atmosphere. The lower the water solubility, the higher the probability of these pollutants to precipitate, partition into the soils and sediments, and bio-concentrate in aquatic organisms. Results from Table 1 and Figure 4, indicate that the compounds studied or detected display very different water-solubility trends. First, DHHB and its by-products are the less soluble compounds of the entire set. This means that they will all be prime candidates to partition into the soils and sediments. Also, chlorination of the parental structure of the compounds always led to solubility decrease in general. All the BP8 species are very soluble, except the trichlorinated analogue. All the pharmaceuticals display high solubility, in particular PAR and its by-products, with very extreme water-solubility. The more prominent solubility of the UV filter BP8 along with the studied pharmaceuticals, especially IMC and its analogues, defines these compounds as very unlikely to partition into the air. However, this feature may be quite problematic, given the fact that these pollutants will subsequently become more difficult to remove from the water phase, leading to the contamination of aquatic organisms. This is particularly important in the case of PAR, one of the most predominant pharmaceuticals consumed in daily life. Considering how extremely soluble it is, the compound and corresponding by-products will become significantly bio-available in the water courses. This important increase in bio-availability, means the compounds will display tendency to bio-magnify in the successive levels of the food-chain, with its eco-toxicological effects becoming inherently more concerning.

As far as the partition coefficients go, they represent a ratio between the amounts of a given chemical in different media, allowing the establishment of a trend for the association potential of the compound to those media. The representation of all the

partition coefficients is given in Figure 5. The octanol-water partition coefficient ($\text{Log } K_{ow}$) allows the identification of the hydrophilic or lipophilic character of the organic pollutants. The octanol-air partition coefficient ($\text{Log } K_{oa}$) describes the partitioning tendency of organic compounds between the atmosphere or gas phase and organic substrates (soil, particles and foliage). The air-water partition coefficient ($\text{Log } K_{aw}$) defines the tendency for a chemical to partition between the atmosphere and the water bodies. The soil organic carbon partition coefficient ($\text{Log } K_{oc}$) determines the mobility of contaminants in the soil. The bio-concentration factor (BCF) describes the ability or propensity of organic pollutants to bio-concentrate in living organisms. Results in Table 1 and Figure 5 show that DHHB and its disinfection by-products are those with the highest $\text{Log } K_{ow}$. This means that, with $\text{Log } K_{ow} > 5$, they are non-polar compounds and will, therefore, have more tendency to absorb to solid phases (soils, sediments and aquatic organisms) and also bio-accumulate in those. IMC, BP8 and their by-products, have $\text{Log } K_{ow}$ values around 4, which means they are moderately polar, and so, will partition towards the aquatic phase and the solid phases. PAR, CBZ and all their related compounds have the lowest $\text{Log } K_{ow}$ values, which was expected, given their significant water solubilities. EE2 and its DBPs display significant $\text{Log } K_{ow}$ values, which means that they will tend to partition more towards solid phases. Also, $\text{Log } K_{ow}$ values generally increase with the degree of chlorination of the compounds. Regarding the results for the $\text{Log } K_{oa}$, the values are very significant for all pollutants, which means that they are unlikely to partition to the gas phase from organic substrates, such as the soil or plants. Earlier, it was concluded, with the help of the K_H and P_v values, that none of the compounds had much proclivity to partition to the atmosphere, since they are all semi-volatile compounds. As expected, the low $\text{Log } K_{aw}$ values confirm those conclusions. The values for the $\text{Log } K_{oc}$ are more significant in the cases of DHHB, IMC and BP8, which indicates that they are less mobile compounds, which was also expected, since they are strongly absorbed towards the solid phases. The lower $\text{Log } K_{aw}$ values found in the case of the pharmaceuticals, means that they are more mobile, especially EE2 and its by-products. As for the BCF values, DHHB, IMC, EE2 and their corresponding analogues, proved to be the vastly more bio-accumulative. As it was approached earlier, in general, highly lipophilic chemicals ($\text{Log } K_{ow} > 5$) will display greater probabilities to bio-concentrate. However, hydrophilic compounds also accumulate in living organisms, although by a different route. In general, highly lipophilicity compounds (higher $\text{Log } K_{ow}$) will be absorbed through the diet and bio-concentrated mainly in the adipose tissues of those beings. Highly hydrophilic chemicals (lower $\text{Log } K_{ow}$), on the other hand, will predominate in the water phase,

which means they will be mainly absorbed through the water, and therefore, bioaccumulate in the aqueous phases of the organisms, such as the blood serum or milk.

4.3.2. Determination of overall persistence and long-range transport potential

Overall persistence (P_{ov}) and long-range transport potential (LRTP) of organic pollutants and by-products, are two important environmental attributes that cannot be determined experimentally. Alternatively, these properties must be determined or estimated by computational methods or tools, according to the partition coefficients ($\log K_{ow}$, $\log K_{oa}$ and $\log K_{aw}$), the half-lives in every environmental compartment (atmosphere, water and soil) and the molecular masses of the compounds. All these parameters were determined by means of the EPI Suite [7, 16, 19-21], and from the input of these properties, the P_{ov} and LRTP were calculated by the OECD Screening Tool using a chemical fate model [17].

Figure 6 presents the results obtained for the P_{ov} of the EPs and their respective DBPs. From the analysis of the results in Figure 6, it is possible to conclude that there are seven problematic contexts: the dichlorinated analogue of DHHB (Cl_2 -DHHB), the dichlorinated form of IMC (Cl_2 -IMC), the dichlorinated form of BP8 (Cl_2 -BP8), EE2 and

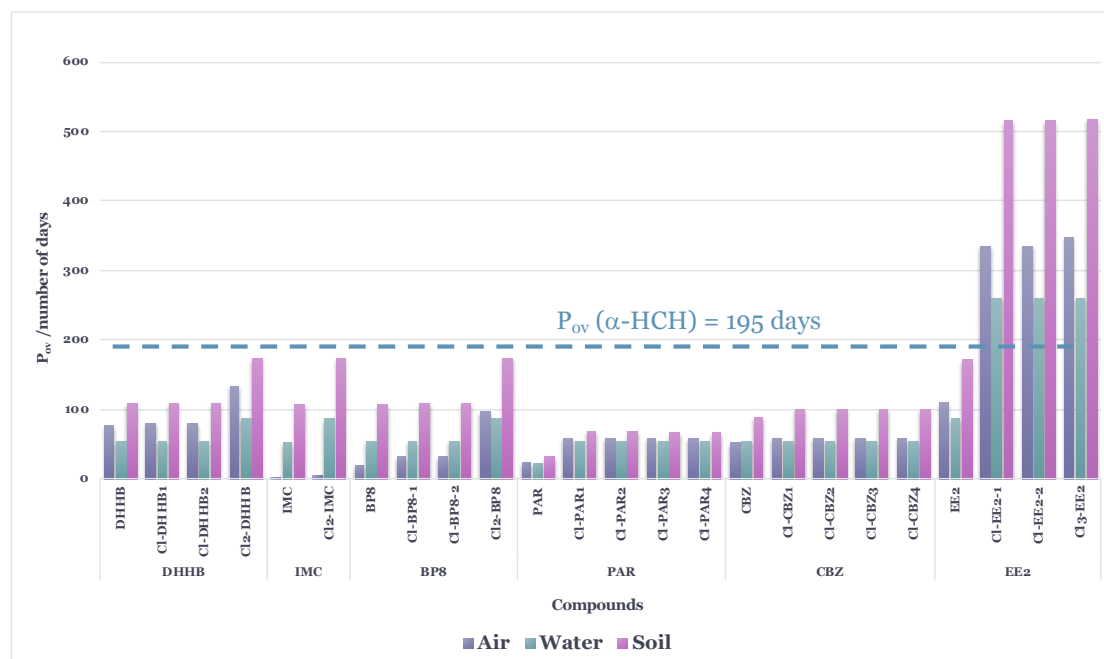


Figure 6. Representation of the overall persistence (P_{ov}) results for every compound studied in this work. Dashed blue line represents the overall persistence of a reference persistent organic pollutant (POP), the α -hexachlorocyclohexane (α -HCH), for analogy purposes.

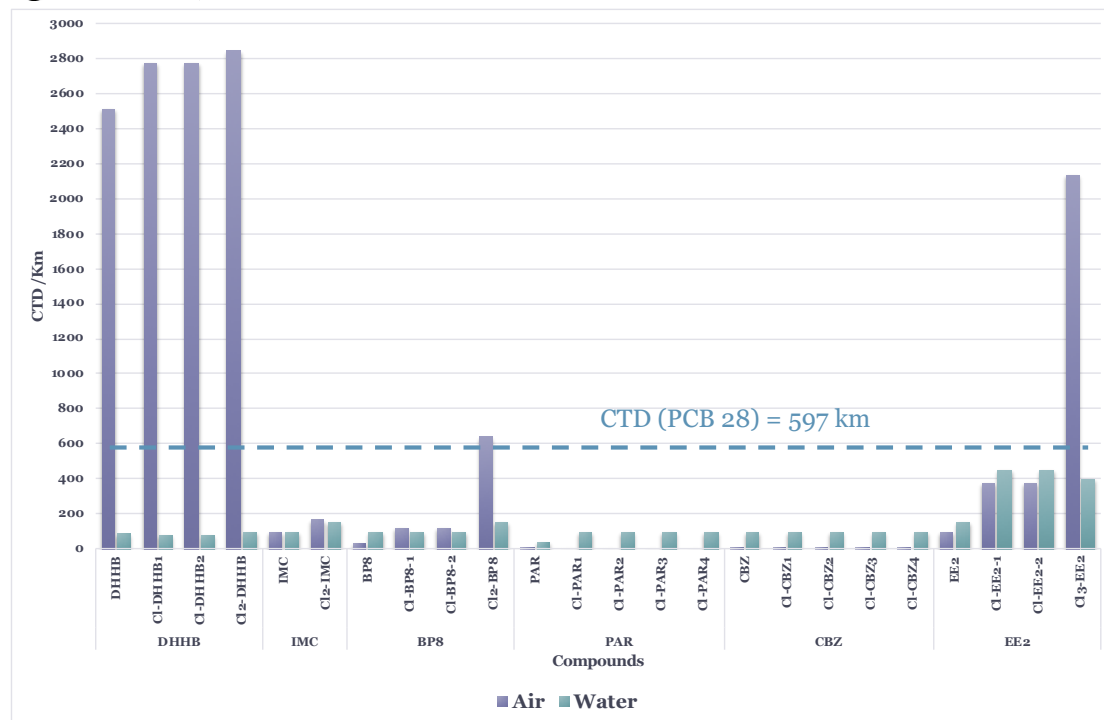


Figure 7. Representation of the long-range transport potential (L RTP), displayed here by the characteristic travel distance (CTD) in kilometres, of all the studied compounds.

all of its DBPs. The dashed blue line in the graphic, refers to the characteristic P_{ov} (195 days) of the known persistent organic pollutant (POP) of reference, α -hexachlorocyclohexane (α -HCH), enlisted in 2009, at the Stockholm Convention for Persistent Organic Pollutants, in the list of POPs to eliminate, given its persistent, bio-accumulative, bio-magnifying and long-range transport abilities [22]. Most of the compounds mentioned above, are dangerously closer to that reference line for persistence, and in the cases of EE2 and its DBPs, they largely surpass that limit. Persistence of Cl_2 -DHBB, Cl_2 -IMC and Cl_2 -BP8 in the soil, is concerningly near the reference line established by α -HCH. The persistence of EE2 in the soil, is also close to that limit line, but all of its detected DBPs display largely more significant persistences than α -HCH, in the atmosphere, water and soil. These by-products may remain more than 300 days in the atmosphere, more than 250 days in the water bodies and more than 500 days in the soil. EE2 is a popular synthetic estrogen, used in oral contraceptives, so its inclusion into the superficial water compartments, including drinking water networks, is very much likely, and in which case, these results are, indeed, very concerning. The influence of molecular attributes such as molecular mass, structure and typical bonds or functional groups present, on persistence of organic pollutants, has been addressed in other studies [7, 20]. According to these, there is a connection between persistence and the molecular structure of the compounds, namely the presence and increase in the number of halogen atoms in the

molecule [7, 20]. The results from the present work seem to support those findings. Additionally, results from Figure 6 show that persistence was also found to be more prominent in the soil, as a general trend. This was to be expected, since there is less mobility in the soil and therefore, less interactions that could lead to compound degradation. The characteristic LRTP of the compounds is presented in two graphical presentations, Figure 7 and 8, namely the characteristic travel distance (CTD) and the superficial travel efficiency (STE), respectively. The blue dashed lines on the graphics represent the values of CTD and TE of a known POP of reference, the polychlorinated biphenyl 28 (PCB-28). CTD indicates the distance to the source of contamination at which the pollutant's concentration decreased to about 38% of its initial amount. STE regards the amount of pollutant that is deposited to the surface media after transportation from the point of contamination. Results from Figure 7 underline eight problematic compounds: once again, all of the DHHB-related compounds show highly significant CTDs in the atmosphere, in other words, once in the air, the compounds will be able to travel between 250 and 2800 km; the dichlorinated form of BP8 in the air, which also surpasses the reference limit of PCB-28; both the dichlorinated analogues of EE2, in the air and water, which are all close to the reference line from PCB-28; and the trichlorinated form of EE2, which largely surpassed the reference limit from PCB-8 in the air, and is close to the line in the case of the aquatic environment. As to how

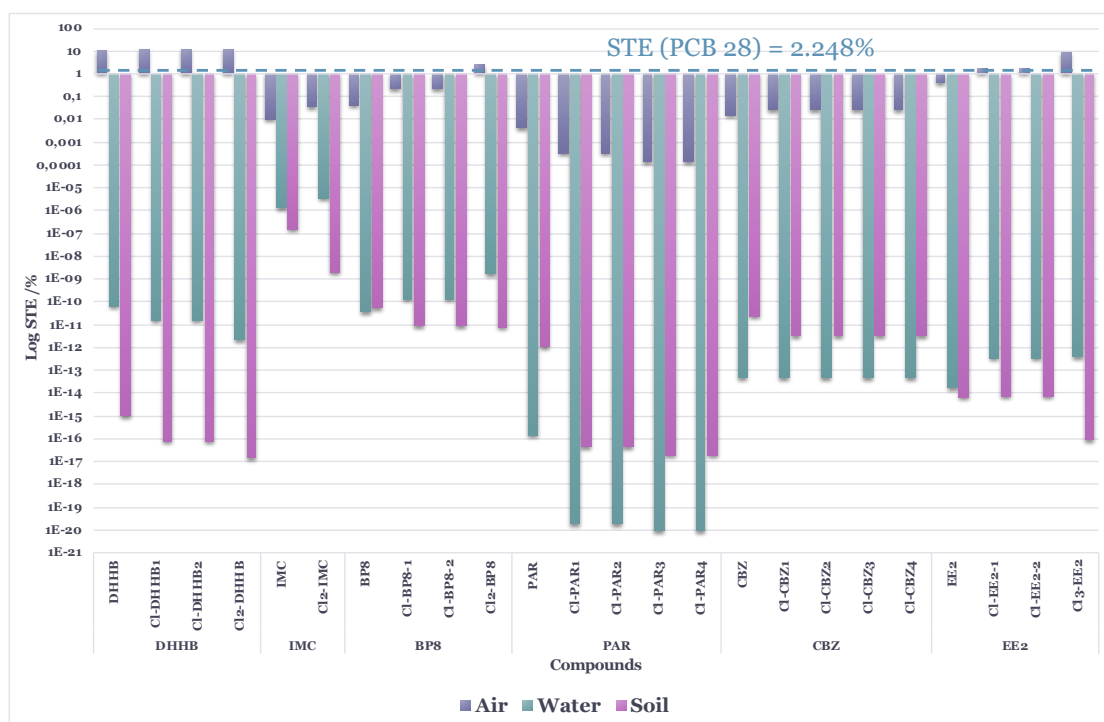


Figure 8. Representation of the superficial travel efficiency (STE) of all the studied compounds. STE units are displayed in logarithmic scale, for better understanding and analogy. The dashed blue line, represents the characteristic STE of another POP of reference, namely the polychlorinated biphenyl 28 (PCB-28).

efficient the pollutants are at being deposited to the surface media, after being transported from the contamination point, there are merely six concerning cases, and all regarding the atmosphere: all the DHHB-related compounds show a 10.79-12.59% efficiency at being deposited in the atmosphere, which is about 8-fold the amount of reference for PCB-28; the dichlorinated form of BP8 also surpassed that reference limit, with 2.40%; both the dichlorinated forms of EE2, with STEs of 1.65%, are also close to the reference value; and the trichlorinated analogue of EE2 displayed an STE of 9.44%, which is also considerably higher than the reference limit presented. Once again, the degree of chlorination seems to play a prominent part in enhancing persistence, transport and expansion, with STE increasing along with the increase of chlorine atoms. This trend was verified in all the compounds, even if the remaining compounds demonstrated STEs considerably lower than the reference limit. Even so, travel efficiency always proved to be more significant in the atmosphere, for all compounds.

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Chapter 5 - Conclusions

5. Conclusions

This work was focused on one paramount objective, the study of the environmental fate and behaviour of important selected EPs, namely, three UV filters (IMC, DHHB and BP8) and three pharmaceuticals (PAR, CBZ and EE2). The environmental compartment fundamentally addressed, were the superficial water bodies, such as river, lakes, and municipal/drinking water, which used for a surplus of applications, including swimming pools. With that being said, study of fate and behaviour involves the approach of different goals or phenomena, such as the evaluation of the degradation reactions of the compounds in such context, which will be influence by two main sources, exposure to water-disinfecting agents and solar radiation. Evaluation of kinetic parameters, such as reaction rate constants and half-life periods, were determined for both degradation scenarios. The formation of disinfection by-products was also assessed, in both those scenarios. Subsequently, the influence of relevant, environmental variables, such as water pH, water temperature, as well as active chlorine and dissolved organic matter concentrations, on the degradation reactions, was also investigated. Last but not least, the physical-chemical properties of all the studied compounds were determined by means of computational models and used to evaluate their environmental persistence and long-range transport potential.

5.1. Determination of kinetic parameters, UV stability and identification of degradation by-products

The UV filter IMC was found to be substantially reactive towards active chlorine. Typically, reactions between organic and inorganic compounds with active chlorine, follow a pseudo-first order reaction profile. In this case, however, the results were found to better suit a pseudo-second order kinetic model. The reaction was overwhelmingly fast, leading to the rate constant $0.042 \pm 0.001 \text{ L mol}^{-1}\text{s}^{-1}$, and a half-life period of 23.8 ± 0.6 seconds. The only DBP detected and identified was the dechlorinated form of IMC. Therefore, chlorination is expected to occur sequentially by electrophilic substitution in the two ortho positions towards the CH_3O - functional group on the benzenic ring of the compound. IMC also displayed considerable photo-stability, although, some degradation was indeed detected, following a first-order reaction model. Photo-degradation was considered to be very slow, yielding a rate constant of $0.00074 \pm 0.00002 \text{ min}^{-1}$ and a half-life period of 938.5 ± 25.4 minutes. No by-products

were detected in the photo-degradation reactions, which is explained by its significant photo-stability.

In the case of UV filter DHHB, disinfection-induced degradation was also determined to be extremely fast, with the compound generating an observed first-order rate constant of $0.0060 \pm 0.0002 \text{ s}^{-1}$ and a half-life period of 116 ± 3 seconds. The only detected DBPs, were one mono-chlorinated and two dichlorinated analogues of the compound. These by-products were found to prevail at lower concentration of chlorine, which indicates that high levels of active chlorine might actually lead to their degradation. DHHB is commercially promoted as outstandingly pho-stable, and results obtained in this study confirm those considerations. Some degradation, though, was indeed detected, around 15% during 6 hours of exposure to artificial solar radiation. This amount was considered negligible. As for the spectroscopic features of the filter, a minor shift in the absorption wavelength of the compound was detected, but also considered negligible, since there is no significant absorption capacity decrease.

In similar fashion, the UV filter BP8 also showed significant reactivity towards active chlorine, with a reaction rate constant of $0.0036 \pm 0.0002 \text{ s}^{-1}$ and a half-life period of 194.12 ± 7.90 seconds. Two DBPs were successfully detected in the disinfection-induced reactions, the mono and dichlorinated forms of BP8. It was concluded that the electrophilic substitution of chlorine occurred, most likely, at the *ortho*- and *para*-positions towards the -OH functional group (or at both ortho positions towards the CH₃O- group). BP8 was also determined to be significantly photo-stable, upon exposure to artificial solar radiation, generating a rate constant of $0.00083 \pm 0.00002 \text{ min}^{-1}$ and a half-life period of 835.12 ± 20.12 minutes. The compound degraded about 25% after 6 hours of irradiation, which is slightly higher than in the case of DHHB. No photo-degradation by-products were also detected, as well as no significant alterations to the UV spectra and absorption capabilities.

As far as the pharmaceuticals are concerned, PAR and EE2 demonstrated to be extremely reactive, whereas CBZ was deemed much more stable. While EE2 was nearly completely degraded, after a period of around 60 seconds, CBZ degraded about 40% after 6 hours of reaction. The disinfection-induced degradation of PAR yielded two monochlorinated analogues of the parental structure. In the case of CBZ, merely one DBP was detected, the monochlorinated form of the compound. As for EE2, two DBPs were detected, the monochlorinated and trichlorinated forms of the original

compound. Regarding photo-induced degradation, all pharmaceuticals were considered stable, with no meaningful effects in UV spectra and absorption capacities.

5.2. Influence of external variables on degradation

Regarding IMC, the most influential variables on degradation, proved to be the active chlorine concentration and water pH. Higher degradation rates were detected throughout the experiments, without DOM in solution and within the acidic-neutral pH range. The overwhelming reactivity of IMC did not appear to be hindered by the presence of DOM in solution, which is known to actively compete for the available active chlorine.

As in the case of IMC, the most influential variables on the degradation of DHHB proved to be the water pH and the active chlorine levels. Higher degradations of the compound were detected at increased concentration of chlorine and lower pH levels, as expected.

Once again, with or without DOM in solution, the water pH and chlorine concentration were determined to be the most significant variables on BP8 degradation. Higher degradation rates were found at higher chlorine concentrations, as expected, but also at lower pH levels. This finding indicates that ionic form of BP8 might predominate in aqueous solution, leading to its increased reaction rates at lower pH and with the less efficient chlorine agent hypochloride anion. DOM was found to be influential in the degradation process, and as it was expected, since its presence led to a somewhat significant decrease in the overall degradation rates, from around 39% (without DOM in solution) to 26% (with DOM in solution).

Just as in the case of the UV filters, the degradation of the selected pharmaceuticals was mainly influenced by the water pH and the concentration of active chlorine in solution. The presence of DOM, however, was found to be influential in generally decreasing the degradation rates of the compounds. CBZ was the only exception to this finding.

5.3. Determination of overall persistence and long-range transport potential

Regarding the overall environmental persistence of the studied compounds, there are essentially seven concerning cases, mostly of DBPs, whose chlorination seems to add disturbing features to the compounds. The most problematic compounds were the dichlorinated form of DHHB, the dichlorinated form of IMC, the dichlorinated analogues of BP8, the compound EE2 and its DBPs. For environmental persistence analogy purposes, the priority organic pollutant α -HCH is used as a reference, with an overall persistence of 195 days. All the compounds just mentioned are very close to that concerning reference value, and in the case of EE2s DBPs, they largely surpass those persistence features. The dichlorinated DBPs of EE2 are expected to persist in the atmosphere nearly 350 days, more than 250 days in the aquatic environment and more than 500 days in the soil. These values are quite disturbing. Studies have reported that molecular features such as molecular mass, bonds and functional groups attached as well as the presence of halogens, has correlation towards environmental persistence and transport. These findings certainly seem to support those reports.

As for environmental transport characteristics, one known POP is used as a reference, the PCB-28, which can travel up to 597 km in the environment and has an environmental distribution efficiency of 2.248%. Once again, in analogy with the reference values, there are eight concerning cases. That is to say, all the DHHB-related compounds, the dichlorinated form of BP8 and the EE2-related compounds. The DHHB compounds, once in the air, can travel between 250-2800 km, the dichlorinated form of BP8 also surpassed the reference limit of PCB-28, the dichlorinated analogues of EE2 are very close to the reference values and the trichlorinated form of EE2 largely surpassed those limits. As far as the surface media deposition is concerned, there are six problematic cases. All the DHHB-related compounds show an efficiency significantly larger than the reference compound. The dichlorinated form of BP8 also displayed a larger efficiency than PCB-28. Both the dichlorinated DBPs of EE2 show a surface efficiency deposition close to the reference value, whereas the trichlorinated DBP largely surpassed the reference limit. As the results demonstrate, the increase in the degree of chlorination seems to significantly increase environmental persistence and transport of organic pollutants. This trend was also verified for those compounds considered below the reference limits.

Appendix

Detection limits of the analytical methods for all compounds studied

Detection limits for the analytical methods for the different compounds were as follows:

Table A1. Detection limits determined for the different analytical methods.

Class	Compound	Acronym	Detection limit
UV filters	hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate	DHHB	1.36 µg/L
	3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate	IMC	0.31 µg/L
	2-hydroxy-4-methoxyphenyl-(2-hydroxyphenyl)-methanone	BP8	0.47 µg/L
Pharmaceuticals	Acetaminophen	PAR	0.43 µg/L
	17 α -Ethinylestradiol	EE2	0.87 µg/L
	Carbamazepine	CBZ	3.43 µg/L

Literature values for the pKa of all studied compounds

Table A2. pKa values for all UV filters and pharmaceuticals studied.

Class	Compound	Acronym	pKa
UV filters	hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate	DHHB	7.29 (strongest acidic); 3.38 (strongest basic)*
	3-methylbutyl-(2E)-3-(4-methoxyphenyl)-acrylate	IMC	-4.8 (strongest basic) [†]
	2-hydroxy-4-methoxyphenyl-(2-hydroxyphenyl)-methanone	BP8	6.78 (strongest acidic); -4.8 (strongest basic) [‡]
Pharmaceuticals	Acetaminophen	PAR	9.38 [§]
	17 α -Ethinylestradiol	EE2	10.33 (strongest acidic); -1.7 (strongest basic)**
	Carbamazepine	CBZ	13.9 ^{††}

* According to the Open Chemistry Database (<https://pubchem.ncbi.nlm.nih.gov/compound/302776-68-7#section=Top>) and Drug Bank (<https://www.drugbank.ca/drugs/DB11269>).
[†] According to Drug Bank (<https://www.drugbank.ca/drugs/DB11207>).
[‡] According to Drug Bank (<https://www.drugbank.ca/drugs/DB11221>).
[§] According to the Open Chemistry Database (<https://pubchem.ncbi.nlm.nih.gov/compound/acetaminophen#section=Stability>).
^{**} According to Drug Bank (<https://www.drugbank.ca/drugs/DB00977>).
^{††} According to the Open Chemistry Database (<https://pubchem.ncbi.nlm.nih.gov/compound/carbamazepine>).

Pre-concentration of the experimental solutions for the determination of potential degradation by-products

Pre-concentration of all the experimental reaction mixtures, prior to the determination of potential degradation by-products by LC-DAD-MS, was carried out for all studied compounds by means of a SPE experimental procedure.

In all cases, two specific types of SPE cartridges were always evaluated, in order to ascertain their efficiency in the overall recovery percentages of the compounds. The cartridges were Oasis HLB 6 mL (500 mg) LP from Waters, and Lichrolut EN 3 mL (200 mg) from Merck. Recovery rates of compound, for both UV filters and pharmaceuticals, ranged from 78.7-83.8%.