OCCURRENCE, FATE AND TOXICITY OF BENZOPHENONE DERIVED COMPOUNDS IN THE AQUEOUS ENVIRONMENT

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Doctoral Dissertation

POJAVNOST, USODA IN TOKSIČNOST DERIVATOV BENZOFENONA V VODNEM OKOLJU

Doktorska disertacija

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Abstract

The increasing use of pharmaceuticals and personal care products (PPCPs) is leading to their widespread occurrence in the environment. An important, yet poorly investigated class of PPCPs are the benzophenone-derived compounds. Although their concentrations in the environment are at trace levels, they are sufficient to induce toxic effects on ecosystems because of chronic exposure. Nevertheless, data regarding their occurrence, cycling and adverse effects in the environment are scarce and their presence in natural waters remains unregulated. The aim of this thesis was to provide information on the occurrence, fate and toxicity of eight benzophenone derivatives in the aquatic environment, which include the pharmaceutical ketoprofen, its phototransformation products 3-ethylbenzophenone and 3-acetylbenzophenone, and five benzophenone-type UV filters (benzophenone, 4-hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 2,4-dihydroxybenzophenone and 2,2'-dihydroxy-4-methoxybenzophenone).

The thesis describes an analytical procedure for determining the selected benzophenone derivatives in surface waters and sediments. The method involves the pre-concentration of water samples by solid phase extraction (SPE) and microwave assisted extraction (MAE) of sediment samples, followed by derivatization and analysis by gas chromatography–mass spectrometry (GC-MS). Different parameters were investigated to achieve optimal method performance. In terms of SPE, the effects of sample volume and pH, elution solvent, and matrix effects were evaluated, using the hydrophilic–lipophilic balanced copolymer as the SPE sorbent (Oasis HLB). The results revealed that sample volume and the type of matrix have no significant influence on the performance of SPE. An acidic pH (pH = 2–3) resulted in equal or higher extraction yields of the investigated compounds. Ethyl acetate (1.8 mL) was chosen as the elution solvent. Overall extraction efficiencies of 91% to 96% were achieved. For sediment extraction, the effect of extraction solvent, temperature, irradiation time and microwave power on MAE yield were investigated. An extraction time of 30 min at 150 °C with the power of 800 W yielded optimal extraction efficiencies of 80% to 99%, where the solvent mixture of methanol and acetone (1:1, v/v) with the addition of 5% (w/w) formic acid was used.

Before the GC-MS analysis of benzophenones, the derivatization process was optimized, where different types and amounts of derivatizing agent, solvents, reaction times and temperatures were studied. Benzophenone, 3-ethylbenzophenone and 3-acetylbenzophenone were derivatized by forming pentafluorobenzyloximes using 300 μL PFBHA in methanol at 60 °C. 3-acetylbenzophenone showed complete derivatization after 1 h, while benzophenone and 3-ethylbenzophenone required further 14 h. The hydroxylated benzophenones were dissolved in 300 μL of ethyl acetate and derivatized by silylation using 30 μL MSTFA (1 h at 60 °C). Derivatized compounds were analyzed by GC-MS with the overall achieved limits of detection of 0.1–1.9 ng L^{-1} for water samples and 0.1–1.4 ng g^{-1} for sediment samples.

The developed method was applied to the analysis of environmental water and sediment samples from Slovenia and Croatia, affected either by recreational activities or by wastewater treatment plant (WWTP) discharges. Ketoprofen (≤ 2900 ng L^{-1}) and its

transformation products (≤ 320 ng L^{-1}) were found in only two rivers (Ljubljanica and Kamniška Bistrica), both receiving WWTP effluents, while UV filters were detected in all 14 surface water samples. The most frequently detected compounds were benzophenone and 2-hydroxy-4-methoxybenzophenone, two of the most commonly used UV filters. They were also the most abundant (≤ 190 ng L^{-1} for benzophenone and ≤ 820 ng L^{-1} for 2-hydroxy-4-methoxybenzophenone). Results showed that WWTP effluents are an important source of ketoprofen, while sunscreen products washed off during bathing contribute significantly to UV filter contamination of surface waters. Importantly, quantifiable amounts of the two ketoprofen transformation products 3-ethylbenzophenone (≤ 320 ng L^{-1}) and 3-acetylbenzophenone (≤ 19 ng L^{-1}) in the aqueous environment are reported for the first time. Ketoprofen and its transformation products were not detected in the sediment samples. Benzophenone levels in sediments were ≤ 820 ng g^{-1}, while concentrations of other compounds were lower (≤ 32 ng g^{-1}). Similar to surface waters, the most frequently detected compounds in sediments were benzophenone and 2-hydroxy-4-methoxybenzophenone. When sediment and water samples are compared, the target compounds favor the aqueous phase, which is in agreement with their physico-chemical properties.

Photostability of benzophenones was investigated by laboratory-scale photodegradation experiments using a medium pressure (MP) UV lamp, which revealed that the degradation of benzophenones follows pseudo-first-order kinetics. Ketoprofen was the most photolabile ($t_{1/2}$ = 0.8 min), while UV filters were more stable with $t_{1/2}$ between 17 h and 99 h. Irradiation by natural sunlight revealed a similar photostability as predicted under laboratory conditions. Solar photodegradation experiments in distilled water, lake and seawater, revealed that the presence of photosensitizers in natural waters significantly affects photodegradation of benzophenones, indicating they also have the potential to undergo direct and indirect photolysis. The exception is ketoprofen, which showed no difference in lake and seawater compared to distilled water ($t_{1/2}$ = 26–28 min), implying direct photodegradation is the dominant elimination process. Other compounds revealed different photolysis rates in natural waters. In lake water, accelerated photodecomposition of benzophenones occurred, which may be attributed to higher levels of nitrate, a known photosensitizer. In contrast, seawater affected photodegradation to different extents, depending on the compound. Due to the presence of several potential photosensitizers in seawater, it was impossible to determine which one is responsible for enhancement or inhibition of photodegradation. Environmental half-lives of 3-ethylbenzophenone and 3-acetylbenzophenone were 10.5–15.4 h and 11.0–95.0 d, respectively, while $t_{1/2}$ values of UV filters were between 3.22 d and 43.3 d. Further, it was shown that the transformation products of ketoprofen, 3-ethylbenzophenone and 3-acetylbenzophenone are actually formed in the environment by exposure of ketoprofen to natural sunlight.

Genotoxicity testing of benzophenone derivatives using the SOS/*umuC* assay on *Salmonella typhimurium* revealed that ketoprofen and its transformation products showed no genotoxic activity, in neither the presence nor absence of a metabolic activation system. UV filters, with the exception of 2,2'-dihydroxy-4-methoxybenzophenone, exhibited weak genotoxic activity in the presence of a metabolic activation system; however, the concentrations tested were much higher than those found in the environment (≥ 125 μg mL^{-1}). The present study addressed genotoxicity of 3-ethylbenzophenone and 3-acetylbenzophenone for the first time. In addition, this is the first report of genotoxicity assessment of benzophenones photodegradation mixtures, produced by MP/UV irradiation. The results revealed that only benzophenone exhibited weak genotoxic activity when exposed to MP/UV irradiation, while other photodegradation mixtures were not genotoxic.

Povzetek

Uporaba zdravilnih učinkovin in sredstev za osebno nego (PPCP) se povečuje, s tem pa se povečujeta tudi njihova prisotnost in razširjenost v okolju. Pomembno skupino PPCP predstavljajo spojine, ki vključujejo benzofenonsko strukturo. Kljub njihovim nizkim koncentracijam v okolju lahko pri kronični izpostavljenosti škodljivo vplivajo na ekosisteme. Podatki o njihovi prisotnosti, kroženju in neželenih učinkih v okolju so po literaturnih podatkih slabo raziskani, zato je bil cilj moje doktorske disertacije raziskati prisotnost v okolju, usodo in toksičnost izbranih derivatov benzofenona, ki vključujejo zdravilno učinkovino ketoprofen, njegova razgradna produkta 3-etilbenzofenon in 3-acetilbenzofenon in pet UV filtrov (benzofenon, 4-hidroksibenzofenon, 2-hidroksi-4-metoksibenzofenon, 2,4-dihidroksibenzofenon in 2,2'-dihidroksi-4-metoksibenzofenon).

V doktorskem delu sem razvila analizno metodo za določanje izbranih benzofenonov v površinskih vodah in sedimentih. Pri vodnih vzorcih sem uporabila ekstrakcijo na trdnem nosilcu (SPE), pri vzorcih sedimenta pa ekstrakcijo z mikrovalovi (MAE). Za določitev optimalnih pogojev analiznega postopka sem preučila različne parametre. Pri SPE sem raziskala vpliv volumna in pH vzorca, vrsto topila za elucijo ter učinek različnih vodnih matric na ekstrakcijo s hidrofilno-lipofilnim sorbentom Oasis HLB. Rezultati so pokazali, da volumen vzorca in vrsta matrice ne vplivata na učinkovitost ekstrakcije, kisel pH (pH = 2–3) pa je prispeval k njenemu večjemu izkoristku. Elucija z etilacetatom (1,8 mL) je bila najučinkovitejša, pri čemer sem dosegla izkoristke SPE od 91 % do 96 %. Pri ekstrakciji sedimenta sem preučila učinek topila, temperature, čas obsevanja in moč mikrovalov na učinkovitost MAE. Optimalni pogoji so vključevali 30 minut ekstrakcije pri 150 °C z močjo 800 W, kjer sem z uporabo zmesi metanola in acetona (1:1, v/v) z dodatkom 5 % (w/w) mravljične kisline dosegla izkoristke od 80 % do 99 %.

Pred analizo vzorcev s plinsko kromatografijo, sklopljeno z masno spektrometrijo (GC-MS), sem morala optimizirati derivatizacijo izbranih spojin, kar je vključevalo izbiro derivatizacijskega sredstva, določitev časa in temperature derivatizacije, vrste topila in količine derivatizacijskega sredstva. Benzofenon, 3-etilbenzofenon in 3-acetilbenzofenon sem pretvorila v pentafluorobenzil oksime z dodatkom 300 µL PFBHA v metanolu pri 60 °C. Za 3-acetilbenzofenon je zadostovala 1 h derivatizacije, medtem ko je bilo za derivatizacijo benzofenona in 3-etilbenzofenona potrebnih še dodatnih 14 h. Hidroksilirane benzofenone sem silirala z MSTFA, in sicer sem vzorce raztopila v 300 µL etilacetata in derivatizirala z dodatkom 30 µL MSTFA (1 h pri 60 °C). Za benzofenone, ki sem jih analizirala z GC-MS, sem dosegla meje detekcije od 0,1 ng L^{-1} do 1,9 ng L^{-1} za vodne vzorce in od 0,1 ng g^{-1} do 1,4 ng g^{-1} za vzorce sedimenta.

Optimizirano analizno metodo sem uporabila za analizo površinskih vod in sedimentov iz Slovenije in Hrvaške. Vzorčila sem na mestih, kjer sem pričakovala povišane vsebnosti izbranih spojin, in sicer za izpusti čistilnih naprav ali v neposredni bližini intenzivnih rekreacijskih dejavnosti v poletnem času. Ketoprofen (≤ 2900 ng L-1) in njegova razgradna produkta (≤ 320 ng L^{-1}) sem določila le v dveh rekah (Ljubljanica in Kamniška Bistrica), ki sta bili vzorčeni za izpusti čistilnih naprav. UV filtri so bili prisotni v vseh 14 površinskih vodah, med njimi sem najpogosteje določila benzofenon in 2-hidroksi-4-metoksibenzofenon, ki sta tudi najbolj razširjena ter pogosto uporabljena UV filtra. Prav

tako sta bila prisotna tudi v najvišjih koncentracijah (benzofenon: ≤ 190 ng L^{-1} in 2-hidroksi-4-metoksibenzofenon: ≤ 820 ng L^{-1}). Rezultati so pokazali, da so izpusti čistilnih naprav pomemben vir ketoprofena, medtem ko je onesnaženost površinskih vod z UV filtri v veliki meri posledica spiranja teh spojin s kože med kopanjem v rekah, jezerih in morju. Prva sem določila prisotnost 3-etilbenzofenona (≤ 320 ng L^{-1}) in 3-acetilbenzofenona (≤ 19 ng L^{-1}) v vodnem okolju. V vzorcih sedimenta sem določila benzofenon v koncentracijah ≤ 820 ng g^{-1}, medtem ko so bile koncentracije ostalih spojin nižje (≤ 32 ng g^{-1}). Tako v vodnih kot tudi v vzorcih sedimentov sem najpogosteje določila benzofenon in 2-hidroksi-4-metoksibenzofenon, medtem ko ketoprofena in njegovih razgradnih produktov v vzorcih sedimentov nisem določila. Na splošno sem izbrane benzofenone pogosteje določila v vodnih vzorcih kot v sedimentih, kar je v skladu z njihovimi fizikalno-kemijskimi lastnostmi.

Izbrane benzofenone sem v laboratorijskih poskusih izpostavila UV sevanju, pri čemer je razgradnja spojin potekala po kinetiki psevdo-prvega reda. Najhitreje je potekala razgradnja ketoprofena ($t_{1/2}$ = 0,8 min), medtem ko so bili UV filtri bolj obstojni z razpolovnim časom med 17 h in 99 h. Pri obsevanju izbranih derivatov benzofenona v destilirani, jezerski in morski vodi z naravno sončno svetlobo sem dokazala, da prisotnost različnih hranil in drugih spojin v okoljskih vodah bistveno vpliva na potek fotorazgradnje benzofenonov. Izjema je ketoprofen, pri katerem nisem zaznala razlike v njegovi razgradnji v jezerski in morski vodi v primerjavi z destilirano vodo ($t_{1/2}$ = 26–28 min), kar nakazuje, da pri razgradnji ketoprofena v okolju prevladuje direktna fotoliza. Razgradnja preostalih benzofenonov je potekala v jezerski in morski vodi različno. Benzofenoni v jezerski vodi so se razgradili hitreje kot v destilirani, kar bi lahko pripisali visoki vsebnosti nitratnih ionov, ki lahko pospešijo fotolizo. Razgradnja benzofenonov v morski vodi pa je potekala različno, vendar zaradi kompleksnosti te matrice nisem uspela določiti, kateri dejavniki vplivajo na povečano oz. zmanjšano fotorazgradnjo. Razpolovni časi so bili 10,5–15,4 h za 3-etilbenzofenon in 11,0–95,0 dni za 3-acetilbenzofenon ter za UV filtre med 3,22 in 43,3 dni. Pri obsevanju ketoprofena s sončno svetlobo sem dokazala nastanek 3-etilbenzofenona in 3-acetilbenzofenona, kar kaže, da slednja razgradna produkta nastajata tudi v okolju pri izpostavljenosti ketoprofena soncu.

Pri testiranju genotoksičnosti benzofenonov z uporabo SOS/*umuC* testa na bakteriji *Salmonella Typhimurium* ketoprofen in njegova razgradna produkta niso pokazali genotoksičnih učinkov. UV filtri, z izjemo 2,2'-dihidroksi-4-metoksibenzofenona, so pokazali šibko genotoksično aktivnost v primeru metabolne aktivacije, vendar le pri višjih koncentracijah (≥ 125 µg mL^{-1}) kot so prisotni v okolju. Genotoksičnost 3-etilbenzofenona in 3-acetilbenzofenona v literaturi doslej še ni bila objavljena. Poleg tega moja študija predstavlja tudi prvo raziskavo genotoksičnosti vodnih raztopin benzofenonov, izpostavljenih UV obsevanju, pri čemer je le benzofenon pokazal rahlo povišano genotoksično aktivnost, medtem ko pri ostalih spojinah nisem zaznala genotoksičnega učinka po UV obsevanju.

Abbreviations

AcBP	=	3-acetylbenzophenone
ACN	=	acetonitrile
ACT	=	acetone
AFB1	=	aflatoxin B1
APCI	=	atmospheric pressure chemical ionization
BCF	=	bioconcentration factor
BP	=	benzophenone
BP-d_5	=	benzophenone-2,3,4,5,6-d_5
BSTFA	=	N,O-bis(trimethylsilyl)trifluoroacetamide
CEC	=	cation exchange capacity
CI	=	chemical ionization
CRM	=	certified reference material
DAD	=	diode array detector
DCM	=	dichloromethane
DH-BP	=	2,4-dihydroxybenzophenone, BP1
DHM-BP	=	2,2'-dihydroxy-4-methoxybenzophenone, BP8
DMSO	=	dimethyl sulfoxide
DNA	=	deoxyribonucleic acid
DOM	=	dissolved organic matter
D_w	=	dry weight
EC_{50}	=	median effective concentration
ECD	=	electron capture detector
EDC	=	endocrine disrupting chemical
EI	=	electron impact
ER	=	estrogenic receptor
ESI	=	electrospray ionization
EtBP	=	3-ethylbenzophenone
EtOAc	=	ethyl acetate
EtOH	=	ethanol
FID	=	flame ionization detector
G	=	bacterial growth rate
GC	=	gas chromatography
H-BP	=	4-hydroxybenzophenone
HEPES	=	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
hER	=	human estrogen receptor
HEX	=	hexane
HLB	=	hidrophylic-lipophylic balance copolymer sorbent
HM-BP	=	2-hydroxy-4-methoxybenzophenone, BP3
HM-BP-d_5	=	2-hydroxy-4-methoxybenzophenone-2’3’4’5’6’-d_5
HPLC	=	high performance liquid chromatography
HRMS	=	high resolution mass spectrometry
INCI	=	International Nomenclature for Cosmetic Ingredients
INN	=	International Nonproprietary Name

iPrBP	=	3-i-propylbenzophenone
IR	=	induction ratio
ISO	=	International standard organization
IT	=	ion trap
IUPAC	=	International Union of Pure and Applied Chemistry
K_H	=	Henry's coefficient, Henry's Law constant
K_{oc}	=	organic carbon partition coefficient
K_{ow}	=	octanol-water partition coefficient
KP	=	ketoprofen
KP-d_3	=	ketoprofen-d_3 (α-methyl-d_3)
LC	=	liquid chromatography
LC_{50}	=	median lethal concentration
LLE	=	liquid-liquid extraction
LOD	=	limit of detection
LOEC	=	lowest observed effect concentration
LOQ	=	limit of quantification
LP	=	low pressure
LPEC	=	lowest positive effect concentration
m/z	=	mass-to-charge ratio
MAE	=	microwave assisted extraction
MeOH	=	methanol
MP	=	medium pressure
MS	=	mass spectrometry
MSD	=	mass selective detector
MS-MS	=	tandem mass spectrometry
MSTFA	=	N-methyl-N-(trimethylsilyl)-trifluoroacetamide
MTBSTA	=	N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide
NADP	=	nicotinamide adenine dinucleotide phosphate
NQNO	=	4-nitroquinoline-N-oxide
NSAID	=	non-steroidal anti-inflammatory drug
OD	=	optical density
ONPG	=	O-nitrophenyl-ß-D-galactopyranoside
PCPs	=	personal care products
PFBHA	=	O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride
pK_a	=	dissociation constant
PLE	=	pressurized liquid extraction
POCIS	=	polar organic chemical integrative sampler
PPCPs	=	pharmaceuticals and personal care products
Q	=	quadrupole
QqLIT	=	quadrupole linear ion trap
QqQ	=	triple quadrupole
QTOF	=	quadrupole time of flight
RP	=	reversed phase
RSD	=	relative standard deviation
rtER	=	rainbow trout estrogen receptor
S9	=	Aroclor 1254 induced rat liver post-mitochondrial fraction
SBSE	=	stir-bar sorptive extraction
SD	=	standard deviation
SDS	=	sodium dodecyl sulphate
SFE	=	supercritical fluid extraction
SIM	=	selective ion monitoring

SPE	=	solid phase extraction
SPME	=	solid phase micro extraction
SRM	=	selected reaction monitoring
$t_{1/2}$	=	half-life
TN	=	total nitrogen
TOC	=	total organic carbon
TOF	=	time of flight
TP	=	transformation product
TWA	=	time-weighted average
UAE	=	ultrasound assisted extraction
UPLC	=	ultra-performance liquid chromatography
UV	=	ultraviolet
WW	=	wastewater
WWTP	=	wastewater treatment plant
λmax	=	absorption maximum

1 Introduction

The environment is contaminated by a number of organic micro-pollutants, released as a result of urban, industrial and agricultural activities. Emerging organic contaminants of special concern are pharmaceuticals and personal care product (PPCP) residues, which include parent compounds, their metabolites and transformation products (TPs). PPCPs are a diverse group of compounds used either as therapeutic drugs or consumer products, applied to the human body e.g., skin care, dental care and hair care products, soaps and cleaners, insect repellents, sunscreen agents and fragrances. They are produced in large quantities on an annual basis throughout the world and their consumption continues to rise. The increasing use of PPCPs is leading to their widespread occurrence in the environment, where they are recognized as compounds of emerging concern.

Although the environmental concentrations of PPCP residues are usually in trace levels (pg–ng L^{-1}), they could have adverse effects on environmental biota, particularly through chronic exposure. The concern over the presence of PPCPs in the environment has increased in recent decades together with the continuous development of more sophisticated and sensitive analytical instruments, resulting in lower detection limits, which has allowed their trace level detection. Consequently, more substances have been detected in various environmental matrices. Nonetheless, the data on the occurrence of emerging contaminants in the aquatic environment is insufficient to provide a reliable environmental risk assessment for these compounds.

Limited knowledge exists about the cycling and fate of PPCPs in the environment. Photodegradation induced by sunlight is an important natural elimination process that has long been considered as a natural way of lowering the concentration of chemicals and thus reducing ecological risk. However, recent studies show that photolysis is not necessarily a purification process, since transformation products formed during these reactions may exhibit even more deleterious effects than the parent compounds. Furthermore, degradation mixtures may contain several TPs, making it impossible to identify them all and to investigate their effects. The transformation products in these mixtures could affect the photolysis rates of each individual TP and act synergistically when exposed to living organisms. Therefore, besides identifying each individual TP, it is important to investigate the effects of degradation mixtures formed during photolytic reactions.

An important class of PPCPs are the benzophenone-derived compounds. The focus of this thesis was to develop analytical methods, which would allow their detection in different environmental compartments at trace levels (low ng L^{-1}) and to evaluate their occurrence in the aquatic environment to provide an assessment of the short- and long-term pollution with these emerging contaminants. Since photodegradation is an important elimination process in natural waters, the study focused on assessing the fate of benzophenones in the aquatic environment under the influence of solar irradiation. Finally, the genotoxic potential of the target benzophenones and their photodegradation mixtures was evaluated in order to assess the risk that these compounds pose to the aquatic ecosystems.

1.1 Benzophenone Derivatives in the Aqueous Environment

1.1.1 Mode of action and application

Benzophenone-derived compounds represent an important class of pharmaceuticals and personal care products. Although they all share a basic chemical skeleton (benzophenone), they include a wide range of compounds with different properties and modes of application, examples being the pharmaceutical ketoprofen and benzophenone-type UV filters. The benzophenone derivatives, which were the focus of this study, are presented in Table 1. These include the pharmaceutical ketoprofen (KP), its phototransformation products 3-ethylbenzophenone (EtBP) and 3-acetylbenzophenone (AcBP), and the UV filters benzophenone (BP), 4-hydroxybenzophenone (H-BP), 2-hydroxy-4-methoxybenzophenone (HM-BP), 2,4-dihydroxybenzophenone (DH-BP) and 2,2'-dihydroxy-4-methoxybenzophenone (DHM-BP).

Ketoprofen is a commonly prescribed therapeutic agent, which belongs to a group of non-steroidal anti-inflammatory drugs (NSAIDs). Due to their analgesic, antipyretic and anti-inflammatory effects, these drugs provide symptomatic relief from pain and swelling in chronic joint disease, which occurs in osteo- and rheumatoid arthritis, as well as in more acute inflammatory conditions such as musculoskeletal injuries and pain. They are also useful in the treatment of postoperative, dental and menstrual pain, and of headaches and migraine. Their therapeutic effects are based on the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2), thereby inhibiting the production of prostaglandins and thromboxanes from arachidonic acid. Ketoprofen is used only as a prescription drug, intended for oral and topical application (Rang, Dale, Ritter, Flower, & G., 2012).

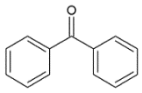
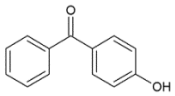
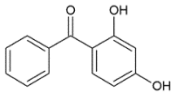
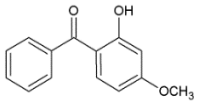
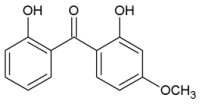
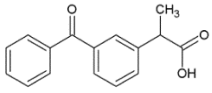
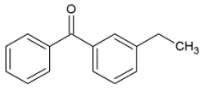
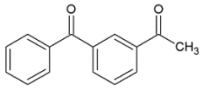
Human consumption of pharmaceuticals worldwide is estimated to be in thousands of tons per year, and continues to rise (McArdell et al., 2006; Ternes, 2001). Ketoprofen has been reported to account for up to 9.5% of the global sales of all NSAIDs (McGettigan & Henry, 2013), however the data about its annual consumption are scarce. In Slovenia, the annual consumption of ketoprofen in 2012 was estimated to be 243 kg a^{-1} (Zupanc, 2013).

UV filters are common ingredients in sunscreens, protecting human skin from the deleterious effects of sunlight. They also serve as UV blockers to enhance product stability of other cosmetic products such as moisturizers, hair sprays, shampoos, hair dyes, lotions, lipsticks and fragrances. Other than in personal care products, these compounds are used as photostabilizers in coatings, adhesives, fabrics, plastic packaging and in car polish (Fent, Zenker, & Rapp, 2010; Giokas, Salvador, & Chisvert, 2007; Jeon, Chung, & Ryu, 2006; Pestotnik, Kosjek, & Heath, 2014; Rodil, Moeder, Altenburger, & Schmitt-Jansen, 2009). In addition, they are used in the manufacture of insecticides, agricultural chemicals and pharmaceuticals, and as photoinitiators, fragrance enhancers, ultraviolet curing agents and flavor ingredients in food industry (Jeon et al., 2006). UV filters used in sunscreens can be either inorganic or organic, and of the twenty-seven UV filters that have been approved by EU Cosmetics Directive for commercial use, twenty-six are organic and only one (titanium dioxide) is inorganic. Inorganic UV filters work as a physical barrier by reflecting and scattering UV light, whereas organic UV filters, including the benzophenone-type, absorb UV light (Champagne, 2009). They are designed to absorb damaging UV rays by blocking UVA (315–400 nm) and UVB (280–315 nm) irradiation.

Due to their unregulated production, the consumption of personal care products is even more difficult to evaluate. It has been reported that the global production of PCPs exceeds 10^6 tons per year (Champagne, 2009). In recent years, the growing awareness of the harm that might be caused by excessive exposure to sunlight has led to an increase in the use of sunscreen products (Li et al., 2007) and as a consequence tones of UV filters are consumed

in Europe annually (Straub, 2002). In addition, different UV filters are often used simultaneously – typically, three to eight individual UV filters are found in sunscreens and cosmetic products (Brausch & Rand, 2011), where their maximum concentrations allowed vary depending upon the compound, accounting for up to 15% of the product's weight (Fent, Zenker, et al., 2010; Salvador & Chisvert, 2005). Since the production of UV filters is unregulated, it remains unclear to what extent these chemicals are produced worldwide.

Table 1: The investigated benzophenone derivatives with their chemical structures, abbreviations and CAS numbers.

IUPAC name, INN / INCI name*	Abbreviation	Chemical structure	CAS Number
Benzophenone	BP		119-61-9
4-hydroxybenzophenone	H-BP		1137-42-4
2,4-dihydroxy- benzophenone, benzophenone-1	DH-BP		131-56-6
2-hydroxy-4-methoxybenzophenone, benzophenone-3	HM-BP		131-57-7
2,2'-dihydroxy-4-methoxybenzophenone benzophenone-8	DHM-BP		131-53-3
2-(3-benzoylphenyl)propanoic acid, Ketoprofen	KP		22071-15-4
3-ethylbenzophenone	EtBP		66067-43-4
3-acetylbenzophenone	AcBP		66067-44-5

* IUPAC: International Union of Pure and Applied Chemistry, INN: International Nonproprietary Name, INCI: International Nomenclature of Cosmetic Ingredients

1.1.2 Sources and exposure pathways

The different modes of application of benzophenone-based PPCPs lead to various pathways by which they can enter the aquatic environment. Pharmaceuticals, such as ketoprofen, are introduced into natural waters through wastewater effluents, which can contain discharges from the pharmaceutical industry, hospitals and private households (Heberer, 2002). In contrast, the most critical source of UV filters is their direct input into lakes, rivers and coastal areas of the sea by wash-off from the skin during swimming, bathing and other water related recreational activities (Giokas et al., 2007).

Figure 1 shows the important pathways by which pharmaceuticals including ketoprofen are released to the aquatic environment. After therapy, ketoprofen is excreted *via* urine and

feces of patients as a mixture of unaltered parent compound and its metabolites through household drains and hospital effluents into municipal sewage. In addition, manufacturing residues from pharmaceutical industry also enter the sewerage system and many unused medicines or those past the expiry date are disposed of down the drain. However, WWTPs are typically designed to eliminate solids, suspended particles, nutrients, and dissolved organic matter, and are not intended for the removal of emerging contaminants such as PPCPs, which means that these compounds can enter surface, ground and finally drinking water (Liu, Ying, Shareef, & Kookana, 2012). Ketoprofen is also used for veterinary purposes, where animal manure can contaminate the soil and ground water (Halling-Sørensen et al., 1998; Heberer, 2002; Kosjek, Heath, & Krbavčič, 2005; Zupanc, 2013). Both EtBP and AcBP have been reported as phototransformation products of ketoprofen when exposed to natural sunlight (Koumaki et al., 2015), which indicates that KP may be their source in the environment.

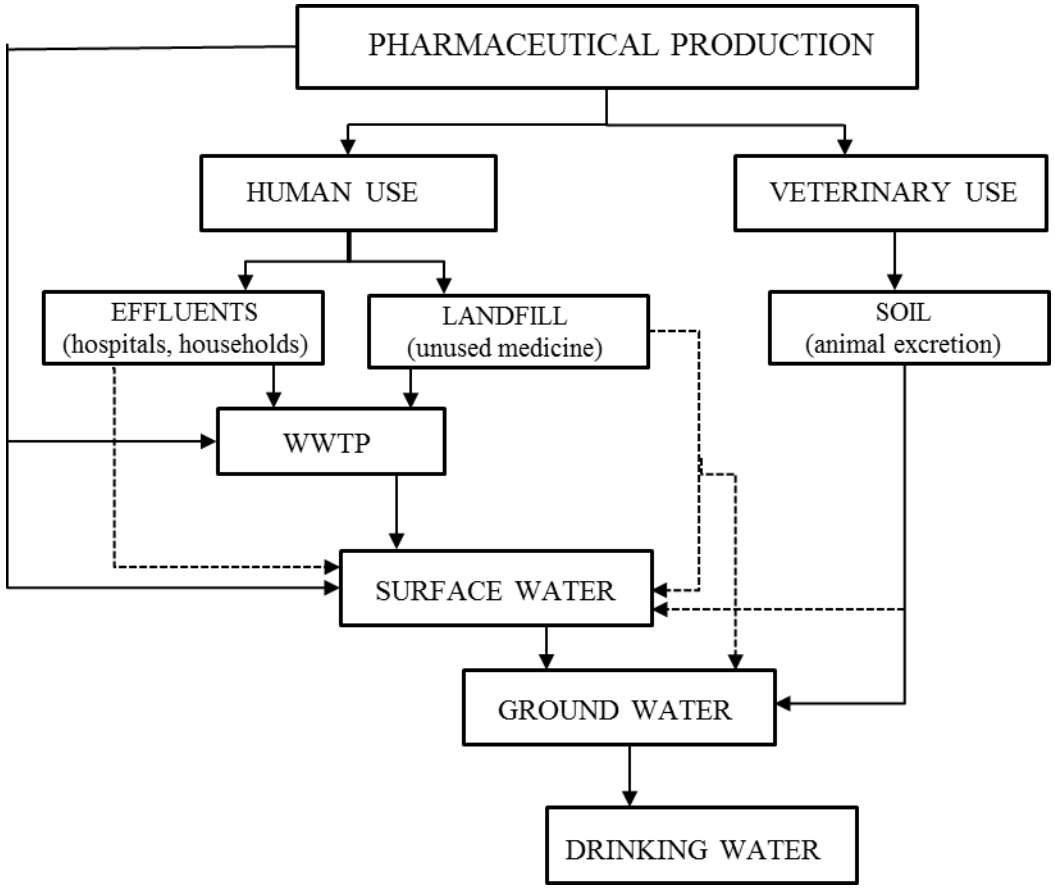


Figure 1: Potential routes by which pharmaceuticals enter the aquatic environment (adapted from Halling-Sørensen et al., 1998 and Heberer, 2002).

Benzophenone-type UV filters enter the aquatic environment through direct and indirect sources (Figure 2). After application of sunscreens and cosmetic products on the skin, UV filters can directly enter surface waters by wash-off from skin during swimming and bathing in lakes, rivers and seas (Balmer, Buser, Müller, & Poiger, 2005; Giokas et al., 2007; Jeon et al., 2006), especially in summer, when these activities mainly take place. Indirect inputs include domestic grey water containing residues from showering and laundering of items (e.g. towels and clothes) that have been in direct contact with UV filters (Balmer et al., 2005; Giokas et al., 2007). Although UV filters are designed to remain on the surface of the skin, they may be partially absorbed into the body after dermal application and further excreted into the municipal sewage (Badia-Fabregat et al., 2012; Balmer et al.,

2005; Rodil et al., 2009). In addition, UV filters may be washed-off from the skin during bathing in swimming pools. They can also reach the environment by industrial wastewater discharge from production facilities or *via* wastewater effluents from WWTPs as a result of their use in products other than PCPs, e.g. textiles, plastics, fabrics and coatings (Balmer et al., 2005; Giokas et al., 2007).

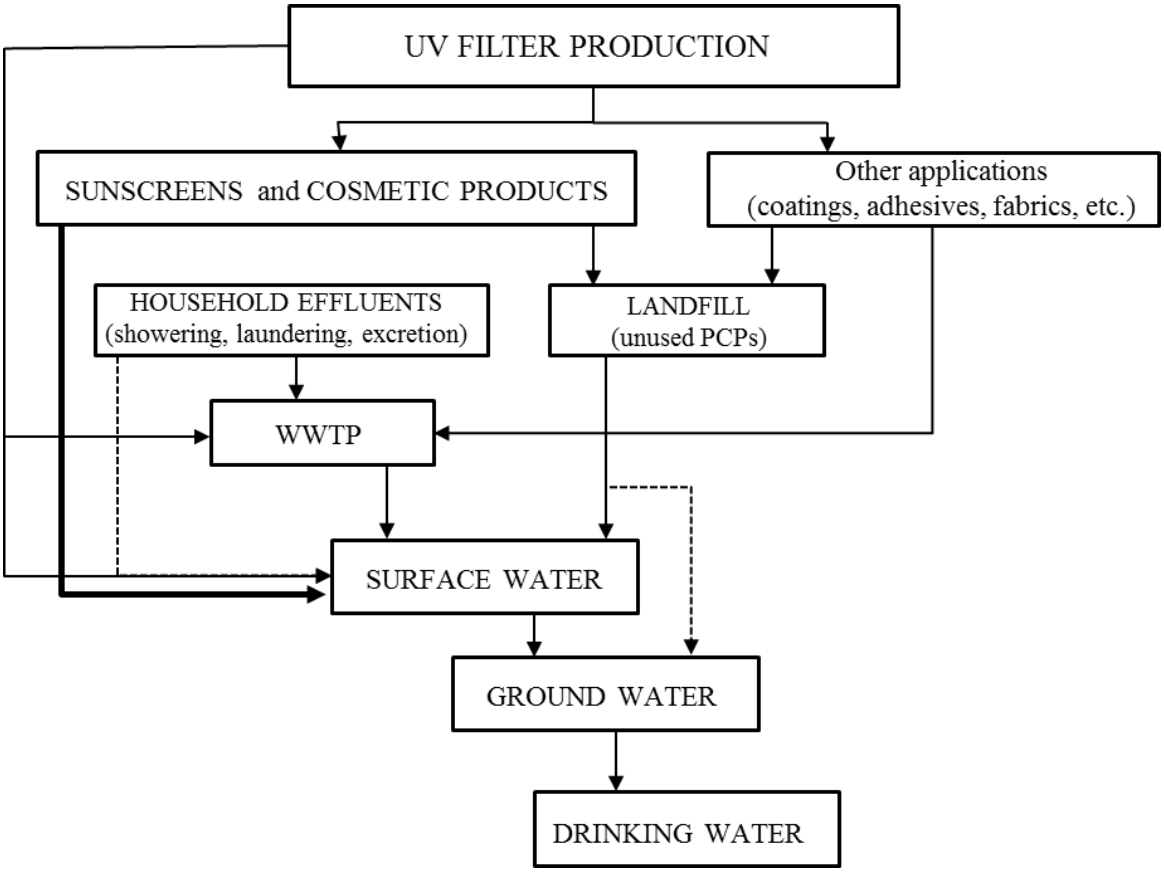


Figure 2: Potential routes by which UV filters reach the aquatic environment (adapted from Balmer et al., 2005 and Giokas et al., 2007).

1.1.3 Physico-chemical properties that influence the environmental behavior

The environmental fate and distribution of PPCPs in the various environmental compartments strongly depend on their chemical structure and physico-chemical properties. These include the dissociation constant (pK_a), solubility, octanol-water partition coefficient (K_{ow}), organic carbon partition coefficient (K_{oc}), bioconcentration factor (BCF), Henry's Law constant (K_H), vapor pressure, absorption maximum (λ_{max}) and atmospheric OH rate (Pestotnik et al., 2014). The available data for the target benzophenone derivatives are given in Table 2.

Selected benzophenones have a solubility in mg L^{-1} (Table 2), allowing them to distribute in the aqueous phase when present in the environment. Their pK_a values, which describe the degree of compound's dissociation at a particular pH, indicate that UV filters are unlikely to dissociate at environmental pHs (6–9). This agrees with their chemical structure, which lacks functional groups that could significantly dissociate at these pHs. In contrast, the pK_a for KP (pK_a = 4.45) indicates that KP is in an ionized form at environmental pHs, resulting in its increased aqueous mobility.

Sorption is a key process controlling the transport of contaminants in the environment.

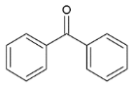
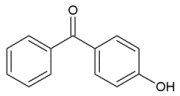
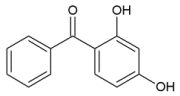
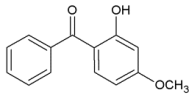
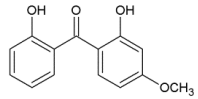
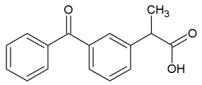
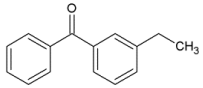
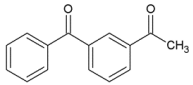
A compound's sorption potential is described by the following parameters: octanol-water partition coefficient (K_{ow}), soil organic carbon–water partitioning coefficient (K_{oc}) and the bioconcentration factor (BCF). The K_{ow} is the ratio of the concentration of a chemical in octanol and in water at equilibrium at a specified temperature. This parameter is used in many environmental studies to determine the fate of chemicals in the environment. This is because polar compounds with lower log K_{ow} values will distribute into the water phase, while compounds with higher log K_{ow} have a greater tendency to bind to organic matter in soils and sediments, and to the fatty tissues of living organisms. The investigated benzophenones have log K_{ow} values between 2.8 and 4.2 (Table 2), which indicates their low to moderate tendency to adsorb to organic particulate matter (ChemIDPlus; HSDB; Sánchez-Brunete, Miguel, Albero, & Tadeo, 2011). The K_{oc} is the ratio of the mass of a chemical that is adsorbed on soil *per* unit mass of organic carbon in the soil *per* equilibrium chemical concentration in solution. These values are useful in predicting the mobility of organic soil contaminants, where generally, higher values correlate to less mobile compounds and *vice versa*. K_{oc} values for the target benzophenones are scant and available only for BP and HM-BP (Table 2). BP is expected to have low to moderate mobility in soil, while K_{oc} of HM-BP indicates a slight mobility (HSDB). BCF is a measure of the ability for water-borne chemical substance to concentrate in the fatty tissue of fish and aquatic organisms relative to its surroundings. The reported BCF values for BP (3.4–12) suggest that its potential for bioconcentration in aquatic organisms is low, while the BCF for HM-BP (33–160) indicates a moderate to high potential for bioconcentration (HSDB).

The transfer between the atmosphere and water bodies is one of the key processes affecting the transport of many organic compounds in the environment. The ratio of a compound's abundance in the gas phase to that in the aqueous phase at equilibrium is referred to as the Henry's Law constant (K_H). Based on the K_H value (1.9×10^{-6}), volatilization from the surface of water and moist soil surfaces is expected to be an important fate process for BP. In contrast, H-BP, HM-BP and KP have K_H values in the 10^{-8} to 10^{-11} atm m^3 $mole^{-1}$ range (Table 2) and as a consequence the fraction removed by volatilization from moist or dry soil or water is not an important fate process (ChemIDPlus; HSDB).

Volatility is defined as the tendency of a substance to vaporize and is directly related to a substance's K_H as well as vapor pressure. If H-BP, HM-BP and KP are released into the air, the estimated vapor pressure, which is around 10^{-5} to 10^{-7} mm Hg at 25 °C (Table 2), indicates that they exist in both the vapor and particulate phases of the atmosphere. Vapor-phase compounds degrade in the atmosphere by photochemically produced hydroxyl radicals and the particulate-phase of benzophenones can be removed from the atmosphere by wet or dry deposition. The vapor pressure of BP (1.93×10^{-3}) indicates that the compound will exist solely as a vapor in the atmosphere (HSDB).

The extent of photodegradation in the environment depends on a compound's ability to absorb radiation in the wavelength region of the solar spectrum (Koumaki et al., 2015). The absorption spectra of the target benzophenones are presented in Figure 3. The amount of UV radiation absorbed by a compound depends on its molecular structure. In general, selected compounds have their absorption maxima (λ_{max}) between 222 and 330 nm (Table 2, Figure 3), and therefore may be susceptible to direct photolysis by sunlight. A compound's potential for indirect photolysis can be evaluated based on a rate coefficient named the atmospheric OH rate, which defines the reaction rate of a compound with hydroxyl radicals (•OH) (Pestotnik et al., 2014). Based on atmospheric rate constants for the compounds listed in Table 2, all available data indicate a similar potential to react with •OH radicals (ChemIDPlus).

Table 2: Physico-chemical properties of the benzophenones (sources: HSDB Database[a], ChemIDPlus Database[b], Kim and Choi, 2014[c]).

Analyte	Chemical structure	Mw	pK_a	Log K_{ow}*	BCF	K_{oc}	Water Solubility (mg L^{-1})	K_H (atm m^3 $mole^{-1}$)	Vapour pressure (mm Hg)	λ_{max}** (nm)	Atm. OH rate (cm^3 $molecule^{-1}$ s^{-1})
BP		182.22	n.a.	3.18*,a,b	3.4–12[a]	430, 517[a]	137[a,b]	1.9×10^{-6} a,b	1.93×10^{-3} a	251**	3.6×10^{-12} a,b
H-BP		198.22	n.a.	2.87* 3.07[b,c]	n.a.	n.a.	406[b]	2.02×10^{-10} b	1.0×10^{-5} b	222, 249, 285**	3.23×10^{-11} b
DH-BP		214.22	7.11[c]	3.17* 2.96[b,c]	n.a.	n.a.	n.a.	n.a.	2.84×10^{-7} c	242, 286, 322**	2×10^{-10} b
HM-BP		228.24	7.56[c]	3.64* 3.79[a,b] 3.52[c]	33-160[a]	2700[a]	69[a,b]	1.5×10^{-8} a,b	1.4×10^{-6} a,b 5.26×10^{-6} c	242, 287, 324**	2×10^{-10} a,b
DHM-BP		244.24	7.11[c]	3.93* 3.82[c]	n.a.	n.a.	n.a.	n.a.	3.73×10^{-6} c	239, 285, 330**	n.a.
KP		254.28	4.45[b]	2.81* 3.12[b]	n.a.	n.a.	51[b]	2.12×10^{-11} b	2.72×10^{-7} b	253**	6.06×10^{-12} b
EtBP		210.27	n.a.	4.17*	n.a.	n.a.	n.a.	n.a.	n.a.	253**	n.a.
AcBP		224.26	n.a.	2.90*	n.a.	n.a.	n.a.	n.a.	n.a.	229, 251**	n.a.

* Values calculated using Advanced Chemistry Development (ADC/Labs) Software (v.12.01); ** Values obtained by measuring the absorbance of benzophenones in ACN at 0.1 M; n.a.: not available

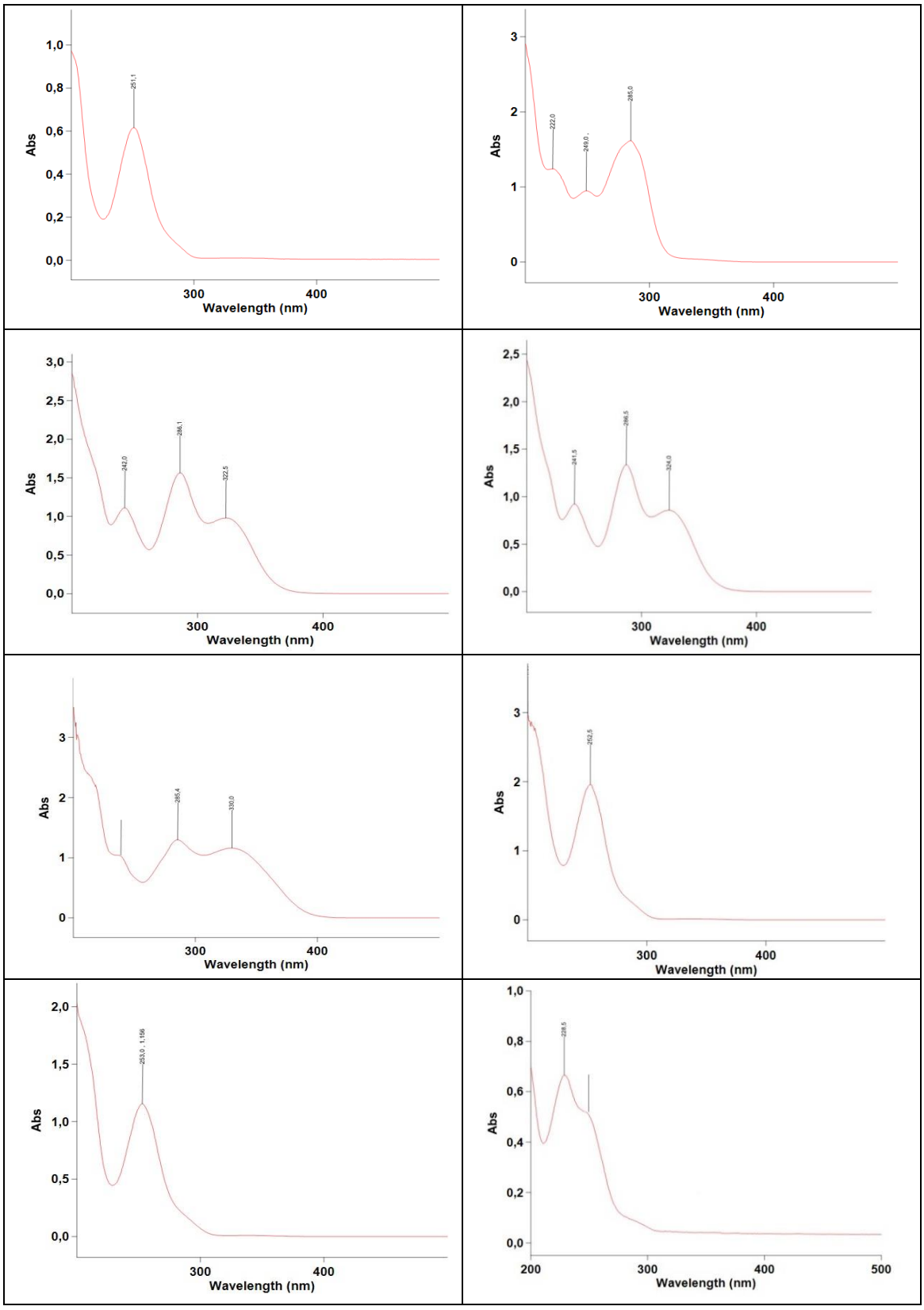


Figure 3: Absorption spectra of the target benzophenones, obtained by measuring the absorbance of benzophenones in ACN at 0.1 M at the Faculty of Chemistry and Chemical Technology, University of Ljubljana.

1.1.4 Occurrence in surface waters and sediments

Only limited data exist on the occurrence of benzophenone derivatives in surface waters. Although the number of investigations in environmental samples has increased over the past decade, the data for a reliable environmental risk assessment are far from sufficient. The available data on the investigated compounds are presented in Table 3. The reported levels of benzophenone-type UV filters in surface waters are in ng L^{-1} (Table 3). Among them, HM-BP has been researched the most, since it is the most commonly used UV filter of benzophenone type, found in numerous sunscreens and cosmetic products (Jeon et al., 2006; Tarazona, Chisvert, León, & Salvador, 2010). HM-BP concentrations found in river and lake water were below 150 ng L^{-1} (Balmer et al., 2005; Cuderman & Heath, 2007; Fent, Zenker, et al., 2010; Jeon et al., 2006; Kameda, Kimura, & Miyazaki, 2011; Kawaguchi et al., 2008; Liu, Liu, Xiong, Yang, & Luan, 2010; Negreira, Rodríguez, Ramil, Rubí, & Cela, 2009a, 2009b; Poiger, Buser, Balmer, Bergqvist, & Müller, 2004; Rodil & Moeder, 2008a; Rodil, Quintana, Lopez-Mahia, Muniategui-Lorenzo, & Prada-Rodriguez, 2008). In seawater, in addition to low levels of HM-BP (Giokas, Sakkas, & Albanis, 2004; Giokas, Sakkas, Albanis, & Lampropoulou, 2005; Lambropoulou, Giokas, Sakkas, Albanis, & Karayannis, 2002), two studies stand out with reported concentrations of 440 ng L^{-1} (Langford & Thomas, 2008) and 3300 ng L^{-1} (Tarazona et al., 2010), but it must be emphasized that the samples were taken during the summer months in enclosed areas with limited exposure to currents, wind or freshwater inputs, allowing the compound to accumulate. In addition, most studies employed collection of grab samples at expected peak pollution (e.g. midday in summer for swimming activities), providing information on peak concentrations (Balmer et al., 2005; Cuderman & Heath, 2007; Farré et al., 2001; Jeon et al., 2006; Kameda et al., 2011; Kosjek et al., 2005; Poiger et al., 2004; Tarazona et al., 2010; Vieno, Tuhkanen, & Kronberg, 2005) and only Fent et al. (2010) applied passive integrative sampling (see Section 1.3.1). Other benzophenone-type UV filters were present in lower concentrations, or their presence was found under the limit of detection (LOD) of the method used (Table 3). The highest concentrations of UV filters were found in bathing waters during summer, when swimming and other recreational activities took place. A pronounced seasonal trend was observed for HM-BP, where sampling was performed before the bathing season in April and again in the peak season in July. Significantly higher levels occurred during the summer (Poiger et al., 2004).

In river water, ketoprofen occurs in concentrations from only a few to several hundred ng L^{-1} depending on the sampling location, i.e. in the vicinity of either a WWTP or a pharmaceutical industry (Antonić & Heath, 2007; Bendz, Paxéus, Ginn, & Loge, 2005; Farré et al., 2001; Kosjek et al., 2005; Tixier, Singer, Oellers, & Müller, 2003; Vieno et al., 2005). EtBP and AcBP could be formed in the environment or during wastewater treatment as ketoprofen transformation products under the influence of sunlight, but their environmental occurrence is yet to be addressed.

Table 3: Reported concentrations of the investigated benzophenone derivatives in surface waters.

Analyte	Matrix	Conc. (ng L^{-1})	Freq.	Location	Reference
BP	River	< 25	0/25	Korea	(Jeon et al., 2006)
		2–82	11/20	Japan	(Kameda et al., 2011)
		23	1/1	Japan	(Kawaguchi et al., 2008)
	Lake	<5	0/6	Korea	(Jeon et al., 2006)
H-BP	River	< 50	0/25	Korea	(Jeon et al., 2006)
		6	1/1	Japan	(Kawaguchi et al., 2008)

	Lake	85	1/6	Korea	(Jeon et al., 2006)
DH-BP	River	47	1/25	Korea	(Jeon et al., 2006)
		< 5	0/1	Japan	(Kawaguchi et al., 2008)
		37	1/1	Spain	(Negreira et al., 2009a)
		< 6–24	1/4	Spain	(Negreira et al., 2009b)
	Lake	< 5	0/6	Korea	(Jeon et al., 2006)
	Sea	< 32–280	1/3	Spain	(Tarazona et al., 2010)
HM-BP	River	< 54–114	1/2	Slovenia	(Cuderman & Heath, 2007)
		< 25	0/25	Korea	(Jeon et al., 2006)
		56–68	2/2	Switzerland	(Fent, Zenker, et al., 2010)
		52	1/1	Spain	(Negreira et al., 2009a)
		4–41	11/20	Japan	(Kameda et al., 2011)
		14	1/1	Japan	(Kawaguchi et al., 2008)
		< 11–30	1/2	Germany	(Rodil & Moeder, 2008a)
		59	1/1	China	(Liu et al., 2010)
		< 8–87	2/4	Spain	(Negreira et al., 2009b)
		< 7–27	1/3	Spain	(Rodil et al., 2008)
	Lake	< 28–85	4/5	Slovenia	(Cuderman & Heath, 2007)
		< 5	0/6	Korea	(Jeon et al., 2006)
		< 2–125	6/7	Switzerland	(Poiger et al., 2004)
		< 2–35	9/11	Switzerland	(Balmer et al., 2005)
		< 11–55	3/4	Germany	(Rodil & Moeder, 2008a)
	Sea	1.8	1/1	Greece	(Giokas et al., 2004)
		6.5–8.2	22	Greece	(Giokas et al., 2005)
		< LOD	0/1	Greece	(Lambropoulou et al., 2002)
		1340–3300	3/3	Spain	(Tarazona et al., 2010)
		< 5–440	17/25	Norway	(Langford & Thomas, 2008)
		< 7–27	0/2	Spain	(Rodil et al., 2008)
DHM-BP	River	< 25	0/25	Korea	(Jeon et al., 2006)
	Lake	< 25	0/6	Korea	(Jeon et al., 2006)
	Sea	< 33	0/3	Spain	(Tarazona et al., 2010)
KP	River	281	1/1	Slovenia	(Antonić & Heath, 2007)
		7–11.5	3/3	Finland	(Vieno et al., 2005)
		10	(C_{max})	Switzerland	(Tixier et al., 2003)
		10–70	3/3	Sweden	(Bendz et al., 2005)
		< 28–300	3/12	Spain	(Farré et al., 2001)
	Lake	< 10	0	Switzerland	(Tixier et al., 2003)

* Conc.: concentration; Freq.: frequency

Sediment, as opposed to water, is less sensitive to factors such as seasonal variations, rainfall and industrial production line schedule, and enables the study of long-term pollution (Antonić & Heath, 2007). In spite of that, the presence of benzophenones in sediments has only recently been addressed. The available data for benzophenones is given in Table 4 and reported concentrations are in the low ng g^{-1} (Antonić & Heath, 2007; Jeon et al., 2006; Kameda et al., 2011; Rodil & Moeder, 2008b; Sánchez-Brunete et al., 2011). The exception is ketoprofen, which was sampled close to a WWTP outflow, and was

present in concentrations up to 320 ng g^{-1} (Antonić & Heath, 2007).

Table 4: Reported levels of target benzophenones in sediment samples.

Analyte	Concentration (ng g^{-1})	Frequency	Location	Reference
BP	1.5–9.7	14/15	Korea	(Jeon et al., 2006)
	2.7–58	19/20	Japan	(Kameda et al., 2011)
H-BP	18	1/15	Korea	(Jeon et al., 2006)
	< 0.23	0/6	Spain	(Sánchez-Brunete et al., 2011)
DH-BP	< 0.1	0/15	Korea	(Jeon et al., 2006)
	< 0.21	0/6	Spain	(Sánchez-Brunete et al., 2011)
HM-BP	< 0.1	0/15	Korea	(Jeon et al., 2006)
	< 0.28	0/6	Spain	(Sánchez-Brunete et al., 2011)
	< 1	0/3	Germany	(Rodil & Moeder, 2008b)
DHM-BP	0.5–2.1	12/15	Korea	(Jeon et al., 2006)
	< 0.14	0/6	Spain	(Sánchez-Brunete et al., 2011)
KP	< 80–320	1/2	Slovenia	(Antonić & Heath, 2007)

1.1.5 Fate in the aqueous environment

Once released into the aqueous environment, PPCPs undergo natural attenuation as a result of a combination of physical (e.g., dispersion, dilution, volatilization, and sorption), biological (e.g., biodegradation and biotransformation), and chemical processes (e.g., hydrolysis, photolysis, and oxidation) (Wang & Lin, 2014). Photodegradation is regarded as potentially the most significant abiotic elimination process for PPCPs present in the aqueous environment (Gago-Ferrero et al., 2012; Lin & Reinhard, 2005; Rodil et al., 2009). The investigated compounds all include a benzophenone skeleton, which allows them to either absorb solar radiation or indirectly react with radicals generated by solar irradiation of photosensitizers in water (Koumaki et al., 2015).

1.1.5.1 Photodegradation

Photochemical reactions that affect the fate of PPCPs in the environment occur as a result of sunlight irradiation, which covers the infrared, visible and UV regions (Figure 4). While UVA (wavelength range 315–400 nm) and UVB (280–315 nm) can reach the earth's surface, solar UVC (200–280 nm) is absorbed by ozone in the stratosphere (Derks, 2010; S. Kim & Choi, 2014). Photodegradation of PPCPs in surface waters is also limited by water turbidity and light attenuation in the upper meters from surface (Matamoros, Duhec, Albaigés, & Bayona, 2009) and depends on the intensity of the solar radiation, eutrophic conditions, depth of the watercourse, latitude and season (Heberer, 2002).

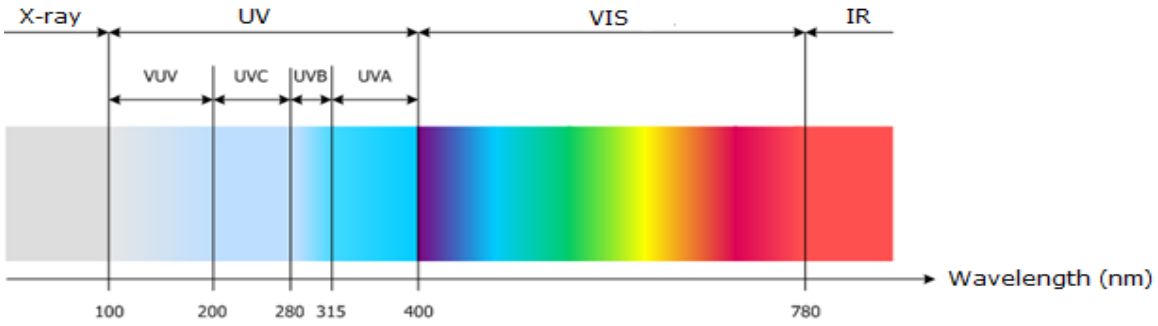


Figure 4: Electromagnetic radiation spectrum (adapted from Derks, 2010).

Direct and indirect photolysis are the primary processes for abiotic transformation of PPCPs in surface waters. Both processes can occur simultaneously and when estimating the environmental half-life of organic compounds, the potential for both should be considered (Lin & Reinhard, 2005). Direct phototransformation occurs when a compound absorbs sunlight, which either enables it to react with the constituents of the water or induce self-decomposition. There must be a good overlap between the absorption spectrum of the compound and the sunlight intensity spectrum (Lin & Reinhard, 2005; Tixier et al., 2003). Indirect photolysis in the environment involves the photodegradation of an organic compound by photosensitizers present in natural waters, such as carbonate, bicarbonate, nitrite, nitrate, sulphate, phosphate, chloride, and dissolved organic matter (DOM) (Derks, 2010; Giokas et al., 2007; Matamoros et al., 2009). Solar irradiation of these substances results in the generation of extremely reactive photooxidants comprising reactive oxygen species such as hydroxyl radicals ($^{\bullet}OH$), peroxyl radicals ($ROO^{\bullet}$), singlet oxygen ($^1O_2$) and other photoreactants, which can react with the compound of interest and enhance photodegradation. Photosensitizers present in surface waters may also compete with the target compounds for light and act as an inner filter hindering light transmission and thereby resulting in reduced rates of photolysis (Derks, 2010; Giokas et al., 2007; Santos, Miranda, & Esteves da Silva, 2012; Silvia Díaz-Cruz, Llorca, & Barceló, 2008). In addition, mechanisms of abiotic degradation produce TPs, which can exhibit a different reactivity and toxicity compared to the parent compound. Therefore, their identification is important when determining the risk that PPCP residues pose to the environment and human health (Giokas et al., 2007; MacManus-Spencer, Tse, Klein, & Kracunas, 2011).

To investigate the photolytic behavior of compounds under controlled laboratory conditions, different sources of radiation may be applied. Among the most commonly used are low (LP) and medium pressure (MP) mercury vapor ultraviolet lamps. The output of a mercury vapor lamp is dependent on the concentration of mercury atoms and thus on the mercury vapor pressure. At a low vapor pressure (near vacuum, 0.13–1.33 Pa) the produced light is monochromatic, which is emitted at a single wavelength (254 nm). When a higher vapor pressure (1.33×10^4–1.33×10^6 Pa) and high operating temperatures (600–900 ºC) are applied, collisions between mercury atoms occur more frequently. The produced light is polychromatic (spectrum between 200–800 nm) and has an overall higher intensity. Therefore, MP lamps emit a broad spectrum of light (Figure 5) coinciding with the absorbance spectrum of many substances, which results in a high photolytic capacity. LP lamps emit ultraviolet light at a single wavelength at 254 nm and, consequently, the direct photolysis of target substances is less effective and the yield of hydroxyl radicals is lower compared to MP lamps. LP lamps also have a significantly lower energy consumption (Derks, 2010). While MP lamps can be useful in estimating environmental fate, LP lamps emit at a wavelength of 254 nm, which is not relevant for predicting the photolytic behavior of compounds in the environment. Because of their radiation, which is in the range of DNA maximum absorption capacity (240–260 nm) (Salgado et al., 2013), the LP lamps are

recognized for their germicidal effects and are commonly used in water treatment for disinfection purposes (Liu, Ying, Shareef, & Kookana, 2011; Szabó et al., 2011).

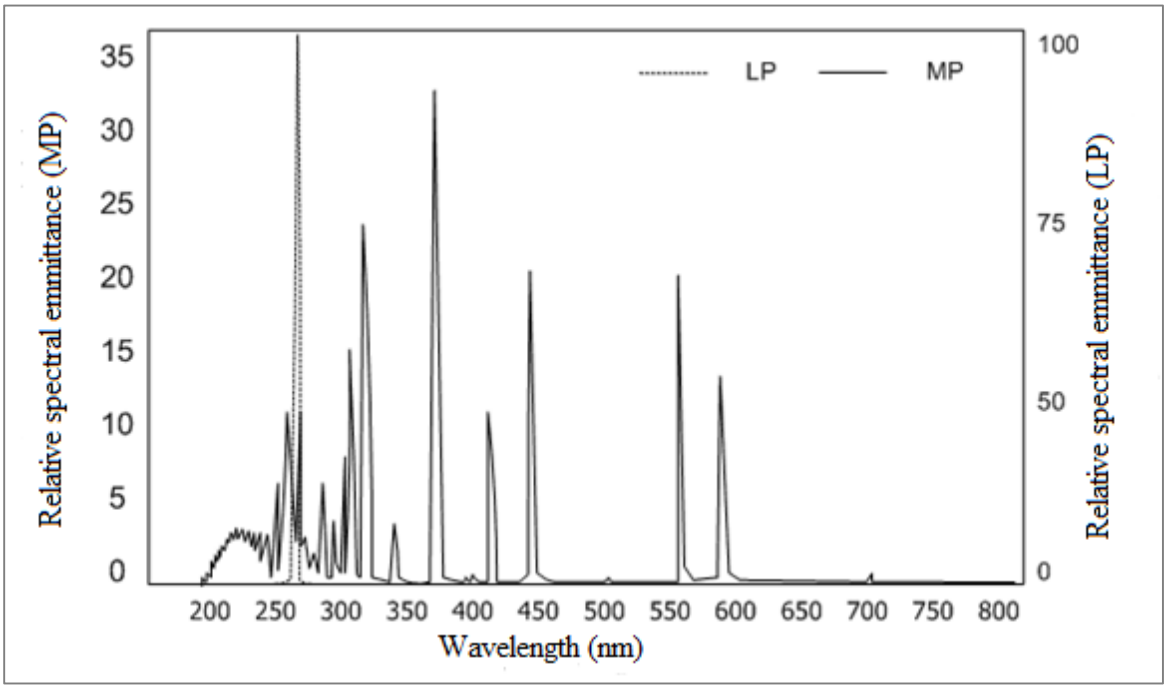


Figure 5: Spectral distribution of LP and MP UV lamps (adapted from Derks, 2010).

Published data concerning environmental photolytic behavior of benzophenone derivatives are scarce. Based on their UV spectra (Figure 3), direct phototransformation of these compounds is possible, while the data with parameters concerning their potential for indirect photodegradation (λ_{max} and atmospheric OH rate) are described in Table 2.

The photolabile character of KP has been previously reported in several studies, performed under different experimental conditions (Kim, Yamashita, & Tanaka, 2009; Kosjek, Perko, Heath, Kralj, & Žigon, 2011; Koumaki et al., 2015; Lin & Reinhard, 2005; Matamoros et al., 2009; Salgado et al., 2013; Szabó et al., 2011). Application of different sources of radiation (xenon and mercury lamps) in various aqueous matrices (Table 5) revealed $t_{1/2}$ values between 0.48 min and 64.2 min (Lin & Reinhard, 2005; Matamoros et al., 2009; Salgado et al., 2013), while natural sunlight irradiation of KP resulted in a $t_{1/2}$ of 2.4 min (Matamoros et al., 2009). After exposure to UV irradiation, ketoprofen rapidly transformed into benzophenone transformation products, with EtBP and AcBP being among them (Kosjek et al., 2011; Szabó et al., 2011). Kosjek at al. (2011) proposed a breakdown pathway for ketoprofen under exposure to UV irradiation, where the chemical structures of twenty-two transformation products were identified, including EtBP and AcBP (Figure 6). All but two of these compounds retained the benzophenone moiety, which raises important issues concerning possible toxic synergistic effects of ketoprofen and its transformation products (Kosjek et al., 2011). Both EtBP and AcBP have been reported as phototransformation products of ketoprofen when exposed to natural sunlight (Koumaki et al., 2015), which indicates that KP may be their source in the environment. Several studies have reported EtBP and AcBP as phototransformation products of KP, but their photolytic behavior is yet to be investigated.

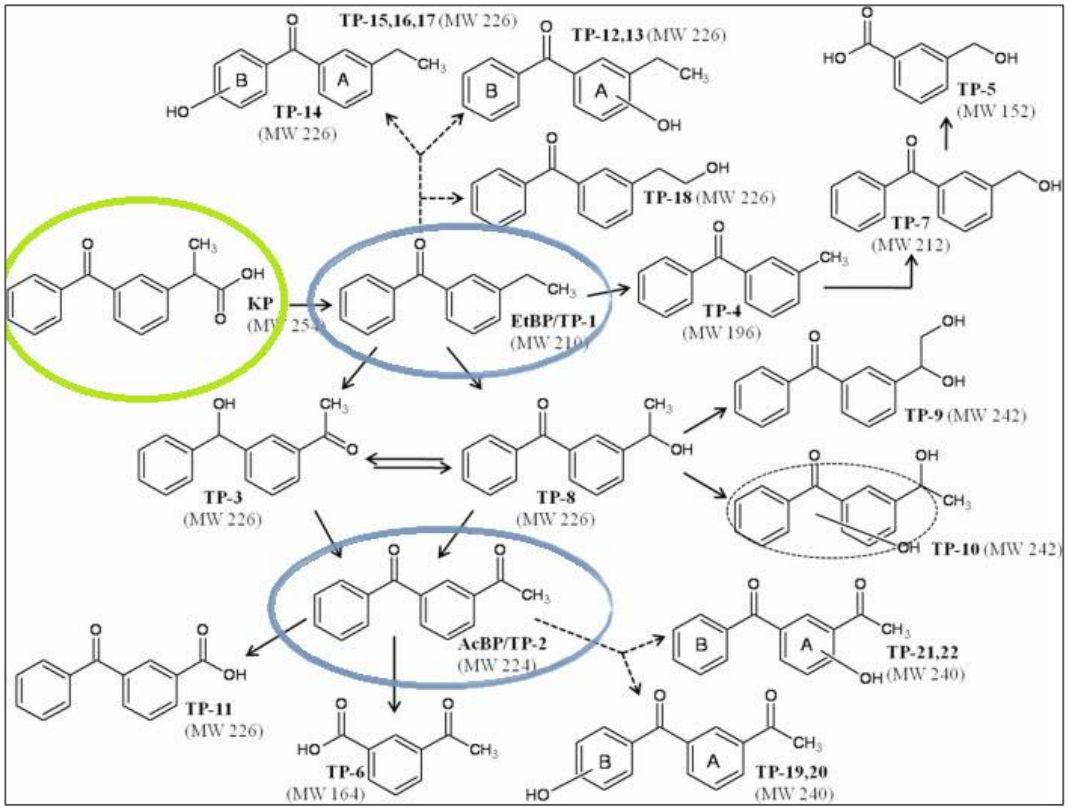


Figure 6: Breakdown pathway of ketoprofen exposed to UV irradiation (adapted from Kosjek et al., 2011).

Until recently, the photostability of benzophenone-type UV filters was studied in order to optimize the dosage and formulation of products to guarantee their long-term protection from UV radiation (Rodil et al., 2009; Silvia Díaz-Cruz et al., 2008), but little effort has gone into examining their environmental fate. Table 5 summarizes photodegradation studies of benzophenones performed under laboratory conditions using UV lamps to simulate solar irradiation in the UV–visible range (290–800 nm) or at 254 nm – a wavelength with germicidal effect. Unfortunately, it is not possible to make a comparison of removal efficiencies, since each study has been performed under different experimental conditions. Studies report the high photostability of HM-BP, which remained almost unaltered (< 10%) when irradiated with different sources of light (Gago-Ferrero et al., 2012; Liu et al., 2011; Rodil et al., 2009), which indicates the potential of HM-BP to persist in the environment. In contrast, DH-BP was almost completely photodegraded after 24 h of irradiation (Gago-Ferrero et al., 2012). The photochemistry of sunscreen products is more complex than the isolated behavior of individual UV filters. Since all sunscreen formulations combine several agents, this needs to be taken into account in further research (Silvia Díaz-Cruz et al., 2008).

The high photostability of benzophenone-type UV filters, the photolabile character of ketoprofen and possible toxic transformation products indicate the need for further research on their fate and behavior in the environment. In addition, any transformation products, formed by abiotic degradation, may exhibit different reactivities and effects compared to the parent compounds (Giokas et al., 2007; MacManus-Spencer et al., 2011), but only a few publications address the formation of phototransformation products of benzophenones (Kosjek et al., 2011; Liu et al., 2011; Rodil et al., 2009; Salgado et al., 2013; Szabó et al., 2011).

Table 5: Available data regarding the photolysis of benzophenones.

Analyte	Irradiation source	Medium	Irradiation time	Removal (%)	$t_{1/2}$ (min)	Reference
HM-BP	Halogen lamp: 290–800 nm; 350 µmol photons m^{-2}	Bidistilled water	70 h	approx. 5%	n.a.	(Rodil et al., 2009)
	UV lamp: 254 nm; 2.3 W m^{-2}	Milli-Q water	540 h	approx. 5%	n.a.	(Liu et al., 2011)
	HP mercury lamp: 290–800 nm	Milli-Q water	50 d	8%	n.a.	
	Xenon lamp; 400 W m^{-2}	HPLC grade water	24 h	0%	n.a.	(Gago-Ferrero et al., 2012)
	Xenon lamp: > 290 nm	Aqueous media	120 min	20%	n.a.	(Silvia Díaz-Cruz et al., 2008)
DH-BP	Xenon lamp; 400 W m^{-2}	HPLC grade water	24 h	> 99%	n.a.	(Gago-Ferrero et al., 2012)
KP	Xenon lamp: 290–700 nm, 250 W m^{-2}	Milli-Q water	n.a.	n.a.	2.4–2.9	(Lin and Reinhard, 2005)
		River water	n.a.	n.a.	4.1 min	
	LP/UV Hg lamp: 254 nm, 1500 mJ cm^{-2}	Milli-Q water	150 min	78%	64.2	(Salgado et al., 2013)
	MP/UV Hg lamp: 200–450 nm	Milli-Q water	n.a.	n.a.	0.27–0.66	
		Unfiltered treated wastewater	n.a.	n.a.	0.66	
		Filtered treated wastewater	n.a.	n.a.	1.23	
	Xenon lamp: 300–800 nm; 507.5 W m^{-2}	Milli-Q water	n.a.	n.a.	0.54	(Matamoros et al., 2009)
		Seawater	n.a.	n.a.	0.54	
		River water	n.a.	n.a.	0.48	
	Sunlight	River water	n.a.	n.a.	2.4	
	Sunlight	Milli-Q water	< 15 min	> 99%	n.a.	(Koumaki et al., 2015)

1.2 Toxicity of Benzophenone Derivatives

Concern over the possible impact of PPCPs on human health and the environment has led to the development of a range of methods for testing their biological effects. Toxicity tests usually follow guidelines, issued by organizations, such as the Organization for Economic Cooperation and Development (OECD), European Environment Agency (EEA) and US Environmental Protection Agency (USEPA) (Timbrell, 2002). Acute and chronic toxicity tests are designed to determine the short- and long-term effects of various chemicals on organisms using a variety of end-points such as survival, reproduction, physiological and biochemical responses. The data obtained by toxicity testing can be used to determine the pathologic effects of contaminants and to provide the dose-response data for comparing exposure levels in the field. Problems occur in extrapolating the data obtained in ecotoxicological tests to field conditions and to species not closely related to the test organisms. These tests provide a useful tool for screening for the most toxic compounds,

but are unable to predict the ecological effects on all species (Kendall et al., 2003).

The widespread use of benzophenone PPCPs has resulted in their release into the aqueous environment and their potential impact on the aquatic ecosystem is of concern. To date, the reported toxicity data are fragmentary and not sufficient to allow an adequate risk assessment.

1.2.1 Ecotoxicity

Reported ecotoxicity data for the investigated benzophenones are scarce, in particular studies concerning chronic toxicity are missing. Table 6 summarizes the available ecotoxicity data for the compounds of interest. Among the target benzophenone derivatives, HM-BP has received the most attention. Sieratowicz et al. (2011) investigated the effect of HM-BP on primary aquatic producers and consumers, the green alga *Desmodesmus subspicatus* and the crustacean *Daphnia magna*. Results revealed the growth inhibition of *D. Subspicatus* with a 72 h IC_{10} value of 0.56 mg L^{-1}, while acute toxicity test using *D. magna* resulted in an EC_{50} of 1.67 mg L^{-1}. Chronic exposure to HM-BP showed no effects on neonate production or the length of adults in *D. magna* reproduction test (Sieratowicz, Kaiser, Behr, Oetken, & Oehlmann, 2011). Fent et al. (2010) report how acute toxicity of selected UV filters, HM-BP among them, increases with their lipophilicity (log K_{ow}) in water flea *D. magna*, since the LC_{50} values decrease from the most polar compound 2-hydroxy-4-methoxybenzophenone-5-sulphonic acid (50 mg L^{-1}) to the most lipophilic 2-ethylhexyl-4-trimethoxycinnamate (0.29 mg L^{-1}). The 48 h LC_{50} value for HM-BP was determined as 1.9 mg L^{-1} (Fent, Kunz, Zenker, & Rapp, 2010). By evaluating reproduction inhibition of the microalgae *Scenedesmus vacuolatus*, the test revealed a 24 h EC_{50} value of 0.36 mg L^{-1} for HM-BP (Rodil et al., 2009). Acute toxicity of DH-BP on the marine calanoid copepod *Acarti tonsa* revealed a 48 h EC_{50} of 2.6 mg L^{-1}, while the authors report that subchronic toxicity of DH-BP in an early life-stage development study largely depends on environmental conditions, such as temperature, salinity, light–dark regime, food and culture density (Kusk, Avdolli, & Wollenberger, 2011).

Toxicity of KP was investigated by performing two bioluminescence inhibition assays using *Vibrio fischerii* and revealed EC_{50} values of 15.6 mg L^{-1} and 19.3 mg L^{-1} in ToxAlert® 100 and Microtox® systems, respectively. The reported toxicity data were comparable to the other NSAIDs investigated (ibuprofen, naproxen, and diclofenac), with EC_{50} values from 10 mg L^{-1} to 40 mg L^{-1} (Farré et al., 2001). Toxicity of EtBP and AcBP is yet to be evaluated, however Illes et al. (2014) investigated the ecotoxicity of KP, subjected to O_3 and O_3/UV treatment, where EtBP and AcBP have been determined as transformation products. Toxicity tests performed using representatives of the three trophic levels of the aquatic ecosystems – producers, consumers and decomposers – *P. subcapitata*, *D. magna* and *V. fischeri*, showed that under experimental conditions the TPs have significantly higher toxicity towards all test organisms than KP itself. Its toxicity correlated to the formation of aromatic TPs including EtBP and AcBP, which indicates that the toxic effects were a result of a mixture of these transformation products and not just KP (Illés et al., 2014).

To summarize, the endpoint values of the investigated benzophenones are in the low mg L^{-1} range, which is more than two orders of magnitude above their environmental concentrations (Balmer et al., 2005; Giokas et al., 2007; Poiger et al., 2004; Rodil & Moeder, 2008a). This suggests that these compounds are not acutely toxic to aquatic organisms at environmental concentrations (Brausch & Rand, 2011). In ecotoxicology, chronic exposure studies are important, since organisms in the environment can be exposed to low concentrations of contaminants over a long period of time (Timbrell, 2002). Therefore, additional chronic toxicity tests should be performed. Furthermore,

benzophenone residues are present in surface waters as mixtures of parent compounds, their metabolites and transformation products, which may pose a risk for sensitive aquatic organisms (Fent, 2010), and the effects of mixtures should also be investigated.

Table 6: Ecotoxicological data of the investigated benzophenones.

Analyte	Assay	Species	Endpoint	Reference
HM-BP	Reproduction inhibition; 24 h	*S. vacuolatus*	EC_{50} = 0.36 mg L^{-1}	(Rodil et al., 2009)
	Acute immobilisation; 48 h	*D. magna*	LC_{50} = 1.9 mg L^{-1}	(Fent, Kunz, et al., 20 10)
	Growth inhibition; 72 h	*D. subspicatus*	IC_{10} = 0.56 mg L^{-1}	(Sieratowicz et al., 2011)
	Acute immobilisation; 48 h	*D. magna*	EC_{50} = 1.67 mg L^{-1}	(Sieratowicz et al., 2011)
DH-BP	Early life-stage development; 48 h	*A. tonsa*	EC_{50} = 2.6 mg L^{-1}	(Kusk et al., 2011)
KP	Bioluminescence inhibition; ToxAlert® 100	*V. fischerii*	EC_{50} = 15.6 mg L^{-1}	(Farré et al., 2001)
	Bioluminescence inhibition; Microtox®	*V. fischerii*	EC_{50} = 19.3 mg L^{-1}	(Farré et al., 2001)

1.2.2 Estrogenic activity

Recent studies indicate that certain benzophenone-type UV filters are potential endocrine disrupting chemicals (EDCs). EDCs are exogenous agents that interfere or disrupt the normal synthesis, secretion, transportation, binding and metabolism of natural hormones, which eventually leads to dysregulation of homeostatic mechanisms, reproduction and development (Koumaki et al., 2015). The data regarding the estrogenic activity of the target benzophenones are presented in Table 7. HM-BP demonstrated *in vitro* estrogenic activity by activation of the human estrogen receptor (hERα) in cell lines (Molina-Molina et al., 2008; Schlumpf et al., 2004; Schreurs, Lanser, Seinen, & van der Burg, 2002). In addition, HM-BP activated the hERβ receptor (Schlumpf et al., 2004; Schreurs et al., 2002). HM-BP and DH-BP showed estrogenic activity in recombinant yeast systems carrying hERα (Gago-Ferrero et al., 2012; Kunz & Fent, 2006b; Kunz, Galicia, & Fent, 2006), while HM-BP exhibited estrogenic activity in a recombinant yeast system expressing the rainbow trout *(O. mykiss)* estrogen receptor (rtERα) (Kunz et al., 2006). HM-BP also induced vitellogenin – a biomarker of exposure to estrogenic compounds – *in vivo* in rainbow trout and Japanese medaka (Coronado et al., 2008).

There are only a few studies concerning the multiple hormonal activities of UV filters in different hormonal receptor systems (Kunz & Fent, 2006b; Schlumpf et al., 2004). Besides estrogenic activity, UV filters may also exert additional activities, both agonistic and antagonistic. Kunz and Fent (2006), studying hormonal activity of HM-BP *in vitro* using recombinant yeast systems carrying either a human estrogen (hERα) or androgen receptor (hAR), were able to elicit multiple hormonal activities, where antiandrogenic activity was the most potent (Kunz & Fent, 2006b). The same authors investigated the estrogenic activity of mixtures of UV filters *in vitro*. Their results show significant interactions occurring in the mixtures, among them also synergistic activity (Kunz & Fent, 2006a). As UV absorbing agents are usually applied as multi-component mixtures (Díaz-Cruz & Barceló, 2009), it is important to assess and understand their activity in such combinations.

Table 7: Estrogenicity assays and toxicological data on the target benzophenones.

Analyte	Assay	Endpoint	EC_{50}	LOEC	Reference
HM-BP	Recombinant yeast assay	hERα activation	12.5 mg L^{-1}	1.6 mg L^{-1}	(Gago-Ferrero et al., 2012)
	Recombinant yeast assay	rtERα activation	5.0 mg L^{-1}	n.a.	(Kunz et al., 2006)
	Recombinant yeast assay	hERα activation	4.2 mg L^{-1}	n.a.	(Kunz et al., 2006)
	Cell line (MCF-7)	hERα activation	0.85 mg L^{-1}	n.a.	(Schlumpf et al., 2004)
	Rainbow trout assay *(O. mykiss)*; 14 d	vitellogenin induction	n.a.	0.75 mg L^{-1}	(Coronado et al., 2008)
	Japanese medaka assay *(O. latipes)*; 21 d	vitellogenin induction	n.a.	0.62 mg L^{-1}	(Coronado et al., 2008)
	Japanese medaka assay *(O. latipes)*; 7 d	vitellogenin induction	n.a.	9.9 mg L^{-1}	(Fent, Kunz, et al., 2010)
DH-BP	Recombinant yeast assay	hERα activation	0.058 mg L^{-1}	0.015 mg L^{-1}	(Gago-Ferrero et al., 2012)
	Recombinant yeast assay	hERα activation	0.17 mg L^{-1}	n.a.	(Kunz et al., 2006)
	Recombinant yeast assay	rtERα activation	0.25 mg L^{-1}	n.a.	(Kunz et al., 2006)
	Fathead minnows *(P. promelas)*; 14 d	vitellogenin induction	n.a.	4.93 mg L^{-1}	(Kunz et al., 2006)

1.2.3 Genotoxicity

Genotoxic chemicals have the ability to induce DNA damage and mutations. They can damage genetic material of humans, as well as that of organisms living in a polluted environment. Hence, the potential effects of increased frequency of DNA damage or mutations must be considered in environmental risk assessment (Hamer et al., 2000). Chronic exposure to low environmental concentrations of these chemicals may affect biodiversity, while in humans it may increase the risk of developing cancer (Žegura, Heath, Černoša, & Filipič, 2009). In addition, continuing degenerative changes of the genetic apparatus, caused by genotoxic chemicals, can lead to the formation of a variety of diseases (Reifferscheid & Heil, 1996).

Data on genotoxic activity of benzophenones are inconclusive. No mutagenic activity of ketoprofen was observed in *Salmonella* strains TA97a, TA100 and TA102 by using the Ames mutagenicity assay, while it was weakly genotoxic in mouse bone marrow cells detected with an *in vivo* sister chromatid exchange assay (Philipose, Singh, Khan, & Giri, 1997). UV filters HM-BP, DH-BP and DHM-BP produced a positive or pseudo-positive initiation activity in the *umu* assay using the *Salmonella typhimurium* TL210 strain. In addition, DH-BP revealed weak tumor promotion activity in the Bhas assay (Nakajima et al., 2006). In another study, DHM-BP increased mutation frequencies in the mouse lymphoma assay, while BP and H-BP showed no effect (Jeon, Sarma, Kim, & Ryu, 2007). In a *S. typhimurium* SOS/*umu* assay, BP showed genotoxic activity only after metabolic activation (Takemoto, Yamazaki, Nakajima, & Yokoi, 2002). Recently, the genotoxicity of fourteen benzophenone derivatives was studied using the SOS/*umu* assay and the lowest positive effect concentrations (LPEC) was from 82 to > 1000 mg L^{-1}. The study focused on the genotoxic potential of benzophenones in terms of chemical substituents contribution.

The results revealed that an increasing hydroxyl substitution on the benzene ring, and ortho, para-di-substitution contribute the most to their genotoxicity (Zhao, Wei, Li, & Du, 2013). Insufficient data means that further research is needed to assess the genotoxicity of benzophenone derivatives as well as of their transformation products, formed in the environment, which is yet to be investigated.

1.2.3.1 SOS/*umuC* assay

Micro-scale toxicity tests based on the use of microorganisms provide the possibility to perform a preliminary screening of the compounds of interest. Among many other tests, micro-scale assays have been developed for determining the carcinogenic and mutagenic properties of chemicals, for example, the Ames test, the SOS chromotest and the SOS/*umuC* assay (Toolaram, Gutiérrez, & Ahlf, 2012). Recently, the SOS/*umuC* assay in particular has served as a high-throughput test, which has been standardized according to ISO 13829 (ISO, 2000) for assessing the genotoxicity of native, un-concentrated water samples. It has been proposed as a screening test for monitoring the toxicity and genotoxicity of surface and wastewater samples (Hamer et al., 2000; Žegura et al., 2009).

The SOS/*umuC* assay has been developed by Oda et al. (1985) for the detection of chemical mutagens and carcinogens (Oda, Nakamura, Oki, Kato, & Shinagawa, 1985). It involves the *S. typhimurium* TA 1535 pSK 1002 bacterial strain, carrying a *lacZ* gene (the structural gene for β-galactosidase) that has been fused to the *umuC* operon (Reifferscheid, Heil, Oda, & Zahn, 1991). The test is based on the ability of genotoxins to induce the expression of the *umuC* gene, which is part of the SOS repair–response system. The fused *umuC-lacZ* gene allows the monitoring of *umuC* expression by measuring β-galactosidase activity in a colometric assay (Hamer et al., 2000; Reifferscheid & Heil, 1996; Reifferscheid et al., 1991; Toolaram et al., 2012).

Cytotoxicity is evaluated by determining the inhibition of bacterial growth spectrophotometrically by measuring absorbance at 600 nm. Bacterial growth (absorbance at 600 nm) and β-galactosidase activity (absorbance at 420 nm) are determined after a two-step incubation phase of the bacteria with the toxin. According to the ISO 13829 protocol, genotoxicity of a chemical is measured by quantifying the induction ratio (IR) as the ratio of β-galactosidase activity of samples and controls in relation to the growth inhibition of the bacteria (Reifferscheid et al., 1991; Toolaram et al., 2012).

The SOS/*umuC* assay has several advantages over other methods. For instance, incubation takes place in a 96-well microplate, allowing a simultaneous screening of a large number of samples, where the results are available within 8 hours (Reifferscheid et al., 1991). In addition, the test is cost effective and highly sensitive (Oda et al., 1985; Reifferscheid et al., 1991).

1.3 Analytical Methodologies for Determination of Benzophenones in the Environment

Detection of PPCPs in the aquatic environment is challenging, since the estimated environmental concentrations are very low, generally in the pg to ng L^{-1} levels. In addition, environmental samples are complex matrices that contain various impurities, which interfere with the analysis of the compounds of interest. Therefore, analytical techniques have to be sensitive enough to enable determination at sufficiently low quantification limits and specific enough to detect target compounds (McArdell et al., 2006).

The critical steps in the analytical procedure for detecting PPCPs in environmental samples are described below and include sampling, sample preparation, separation,

detection and data analysis as well as quality assurance. The choice of an analytical method depends on the properties of the analyte, the purpose of the study, and available instrumentation.

1.3.1 Sampling

Sampling is usually the first step in any analytical procedure. The occurrence of PPCPs in the aquatic environment depends on the following factors: time (seasonal, weekly, and daily variations), climatic conditions (rain, dry periods) and means of sampling (grab sample, time/flow proportional sampling, and passive sampling). Accordingly, it is very important to know exactly what kind of sampling method we wish to apply (Buchberger, 2011; McArdell et al., 2006).

Depending on the purpose of the study, different sampling strategies for aqueous matrices can be applied. Grab sampling provides information on the concentration of the target analytes at an exact time, however it does not take into account fluctuations in concentration with time, weather and other variations. Composite sampling is used when calculating loads or mass fluxes, since it is constructed of multiple individual samples taken over a preselected period. Time proportional samples are collected in a defined sampling period at uniform time intervals, e.g. every hour in 24 h period. Flow proportional sampling considers fluctuations in flow rate with time and is utilized to quantitatively assess average water loads. To obtain an average sample, time proportional sampling is suitable for evaluating constant discharges, while flow proportional sampling is more appropriate for variable discharges (McArdell et al., 2006). Passive sampling provides information about time-weighted average concentrations (TWA) and is ideal for sampling of compounds, of which the concentration varies significantly over time. In recent years, a passive sampling device known as a Polar Organic Chemical Integrative Sampler (POCIS) has been introduced. The device consists of a sorbent phase trapped within two thin polyethersulfone membranes. Compounds then diffuse from the water through the membrane and are adsorbed on the sorbent phase. Back in the laboratory, the analytes are eluted from the sorbent using a suitable solvent and analyzed, resulting in TWA concentration (Buchberger, 2011; Richardson & Ternes, 2011).

When monitoring organic pollutants in solid environmental matrices (e.g. sediment), it is important to consider the non-homogenous character of the matrix (Diaz-Cruz, López de Alda, & Barceló, 2003). It is generally accepted that silt and clay with a particle size of < 63 μm accumulate greater concentrations of contaminants than course particles, since fine-grained particles exhibit properties suitable for different physico-chemical sorption and the ion exchange of contaminants (Förstner, 2004). The most frequently used sampling devices for sampling sediments are grab samplers and corers. Different types of corers (e.g. box corers or multi-corers) are used when detailed information on the spatial distribution of analytes is required (Diaz-Cruz et al., 2003). Considering the desired time of pollution assessment, different sampling strategies can be applied. Surface sediment layers provide information on current contamination, while dark layers directly underneath the upper oxidized layer (approximately 1–3 cm depth) are representative of the pollution situation over the last few years. To evaluate historical pollution, vertical profiles using a gravity or valve corer should be used. A core profile of 1 m can cover a historical period of at least 200 years, depending on the sedimentation rate of the investigated water body (Förstner, 2004). Considering the material used for the sampling devices, stainless steel provides the best results (Diaz-Cruz et al., 2003).

For the analysis of benzophenone derivatives in water, different sampling strategies have been employed (Table 8). Most studies applied periodic collection of grab samples at expected peak pollution (e.g. midday in summer for swimming activities), showing the

extent of pollution by providing information on peak concentrations (Balmer et al., 2005; Jeon et al., 2006; Kameda et al., 2011; Kosjek et al., 2005; Poiger et al., 2004; Tarazona et al., 2010). More rarely, to take into account fluctuations with time and provide information about average loads of contaminants, studies employed passive sampling by POCIS (Fent, Zenker, et al., 2010; Zenker, Schmutz, & Fent, 2008). In the few studies investigating benzophenones in sediments, they mostly took grab samples to determine current pollution levels (Antonić & Heath, 2007; Jeon et al., 2006; Kameda et al., 2011). Only Sánchez-Brunete et al. (2011) used a corer to obtain depth profiles of benzophenone-type UV filters in sediment samples.

Table 8: Sampling techniques, employed in the environmental analysis of benzophenones.

Matrix	Sampling	Analyte	Reference
Water	Grab sampler	KP, H-BP, BP, HM-BP, DH-BP, DHM-BP	(Balmer et al., 2005; Jeon et al., 2006; Kameda et al., 2011; Kosjek et al., 2005; Poiger et al., 2004; Tarazona et al., 2010)
	POCIS	HM-BP, DH-BP	(Fent, Zenker, et al., 2010; Zenker et al., 2008).
Sediment	Grab sampler	KP, BP, H-BP, HM-BP, DH-BP, DHM-BP	(Antonić & Heath, 2007; Jeon et al., 2006; Kameda et al., 2011)
	Corer	H-BP, HM-BP, DH-BP, DHM-BP	(Sánchez-Brunete et al., 2011)

1.3.2 Extraction

Detection of PPCPs in environmental samples is often not possible without a sample preparation step, which includes extraction of the target analytes from the matrix and removal of unwanted compounds, concentration, and purification.

1.3.2.1 Extraction of aqueous samples

Various extractions methods for concentrating target analytes from aqueous matrices are available, including liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), solid phase extraction (SPE), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE). The choice of the technique depends on the type of sample and analyte, and the available equipment (Giokas et al., 2007; McArdell et al., 2006).

One of the most widely used methods for extraction of the analytes from aqueous matrices is SPE (Buchberger, 2011; Richardson & Ternes, 2011), which is described in details below. The many advantages of this method have led to the fact that SPE has gradually replaced classical LLE and has become one of the most widely used extraction techniques for aqueous matrices (Lambropoulou, 2010). Compared to LLE, it enables higher recoveries, improved selectivity, specificity and reproducibility, elimination of emulsions, less organic solvent usage, shorter sample preparation time, easier operation and the possibility of automation (Pavlovic, Babic, Horvat, & Kastelan-Macan, 2007). The alternatives to SPE are sorptive extraction techniques, such as SPME, SBSE and several related variants.

Sorptive extraction is based on establishing a partition equilibrium between the analytes

in the aqueous phase and a solid sorbent. In SPME, analytes are trapped on the sorbent, which is present as a coating on a fiber. In case of SBSE, a magnetic stir bar is coated with the sorbent, resulting in a higher volume and larger surface area of the sorbent phase, and consequently improved sensitivity compared to SPME. However, there is a limited amount of commercially available sorptive materials for SBSE, which would enable extraction of polar analytes (Buchberger, 2011).

1.3.2.1.1 Solid phase extraction

Solid phase extraction is performed by passing the samples through pre-conditioned cartridges, containing one or more SPE sorbents, on which the target compound is adsorbed. The critical steps in SPE include conditioning of the cartridges, sample loading, washing the sorbent (to remove interferences), air-drying the sorbent, and elution of the analytes (McArdell et al., 2006).

The selection of suitable sorbents in SPE cartridges is crucial as it can control parameters such as selectivity, affinity and capacity. The choice depends on the characteristics of the investigated compounds, the interactions of the chosen sorbent with the functional groups of the analytes (e.g. dispersion, dipole/dipole, ion/dipole, hydrophobic interaction, ion exchange, etc.) and on the type of sample matrix and its interactions with both the sorbent and the analytes (McArdell et al., 2006; Pavlovic et al., 2007). The most commonly used SPE materials can be classified according to their structure into the following groups (McArdell et al., 2006):

- graphitized
  - graphitized carbon black (e.g. Carbopack B®, Carbograph®)
- silica based
  - reversed phase (e.g. RP-C_8, RP-C_{18})
  - reversed phase endcapped (e.g. with trimethylchlorosilane)
  - strong anionic exchange (SAX) (e.g. benzene sulfonic acid)
  - strong cationic exchange (SCX) (e.g. trimethyl-ammonium)
- copolymers
  - polystyrene-divinylbenzene copolymer (LiChrolut® EN, Strata® X)
  - hydroxlated-polystyrene-divinylbenzene copolymer (Isolute® ENV+)
  - divinylbenzene-N-vinylpyrrolidone copolymer (OASIS® HLB)
- mixed phases
  - RP-C_8 and SCX
  - sulfonic acid anion divinylbenzene-N-vinylpyrrolidone copolymer (OASIS® MCX)
  - quaternary amine cation divinylbenzene-N-vinylpyrrolidone copolymer (Oasis® MAX)

Non-polar substances can be extracted by using a reversed-phase material such as alkyl-modified silica or polymer-based materials (Buchberger, 2007). For aromatic analytes graphitized carbon black can be used, which also contains positively charged oxonium groups that are weak anion exchangers. Anionic and cationic organic compounds can be extracted using ion exchange materials (SAX, SCX) and with mixed phases (e.g. RP-C_8 combined with SCX). For the extraction of non-polar to quite-polar analytes, copolymer materials e.g. polystyrene-divinylbenzene copolymer, hydroxlated-polystyrene-divinylbenzene copolymer, and divinylbenzene-N-vinylpyrrolidone copolymer are suitable phases of choice (McArdell et al., 2006). Extraction of polar analytes from aqueous matrices with SPE is more challenging (Buchberger, 2007). Since many PPCPs present in the environment have polar properties, mixed-mode SPE materials exhibiting both hydrophobic and ion-exchange properties are a valuable alternative (Buchberger, 2011).

Mixed-mode materials can be used for simultaneous SPE of samples containing various compounds, since non-polar analytes would be extracted by hydrophobic interactions and ionized analytes would interact *via* ion exchange mechanisms e.g. copolymer with cationic exchanger groups – sulfonated divinylbenzene-N-vinylpyrrolidone copolymer. In addition, different copolymers are widely used as SPE sorbents, for example the copolymer of divinylbenzene and vinylpyrrolidone, which has been commercialized under the trade name Oasis HLB (Waters Corp.). Nowadays, this is one of the most widely used sorbents with hydrophilic-hydrophobic balance material, which is suitable for multi-class analysis of PPCPs in aquatic environmental samples (Buchberger, 2007, 2011; Richardson & Ternes, 2011).

After percolation, the SPE cartridges are usually dried and the analytes desorbed from the sorbent by elution with organic solvents. Besides the sorbent material, there are several other important parameters: sample flow rate, wash-up and choice of elution solvent (Lambropoulou, 2010; McArdell et al., 2006). To ensure a sufficient sorption of the analytes to the sorbent, the flow rate of the samples passing through the cartridges should be sufficiently low. The elution solvent has to be chosen in consideration to the physico-chemical properties of the analyte, sample matrix and sorbent material. It is important to choose an appropriate amount of solvent, which has to be of sufficient volume for the target analyte to separate from the sorbent as well as low enough to enable good limits of detection and sensitivity. The volume of the elution solvent depends on the particular analyte, its affinity for the sorbent, and on the strength of the eluting solvent used to elute it from the sorbent (McArdell et al., 2006; Pavlovic et al., 2007).

Different analytical methods have been employed for determining target benzophenone derivatives in environmental samples, yielding comparable extraction efficiencies (Table 9). The most commonly used technique for isolation and pre-concentration of water samples is SPE (Balmer et al., 2005; Cuderman & Heath, 2007; Farré et al., 2001; Giokas et al., 2004; Kosjek et al., 2005; Negreira et al., 2009b; Poiger et al., 2004; Rodil et al., 2008; Vieno et al., 2005), although SPME (Lambropoulou et al., 2002; Liu et al., 2010; Negreira et al., 2009a) and SBSE (Migaku Kawaguchi et al., 2006; Kawaguchi et al., 2008; Rodil & Moeder, 2008a) have also been used. LLE is only rarely applied, with reported extraction efficiencies of 62–114% (Jeon et al., 2006).

Table 9: Extraction techniques applied to the environmental analysis of the target benzophenones.

Extraction Technique	Analyte	Extraction Efficiency (%)	Reference
SPE	KP, H-BP, HM-BP, DH-BP, DHM-BP	64–108	(Balmer et al., 2005; Cuderman & Heath, 2007; Farré et al., 2001; Giokas et al., 2004; Kosjek et al., 2005; Negreira et al., 2009b; Poiger et al., 2004; Rodil et al., 2008; Vieno et al., 2005)
SPME	HM-BP, DH-BP	74–108	(Lambropoulou et al., 2002; Liu et al., 2010; Negreira et al., 2009a)
SBSE	BP, H-BP, HM-BP, DH-BP	63–128	(Kawaguchi et al., 2006; Kawaguchi et al., 2008 Rodil & Moeder, 2008)
LLE	BP, H-BP, HM-BP, DH-BP, DHM-BP	62–114	(Jeon et al., 2006)

1.3.2.2 Extraction of solid samples

Extraction of organic compounds from solid environmental samples has been mostly performed by Soxhlet extraction, sonication and shake-flask extraction (Dean & Xiong, 2000). Soxhlet extraction requires large volumes of organic solvents, which are refluxed through the solid sample for up to 24 h, making the method time consuming and environmentally unfriendly. Ultrasonic assisted extraction (UAE) is fast and cost-effective, but not able to produce high recoveries for all classes of analytes. For these reasons, alternative extraction approaches have been developed, implementing new instrumental techniques, such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and microwave assisted extraction (MAE) (Camel, 2000; Dean & Xiong, 2000).

Supercritical fluid extraction is based on the use of a supercritical fluid (typically CO_2) at temperatures and pressures above their critical points ($T > 31.1$ °C, $p > 74.8$ atm), while PLE operates using liquids at temperatures above their normal boiling points and pressures sufficient to keep the extracting fluid in the liquid state. In contrast to SFE, PLE is completely automated, allowing the sequential extraction of up to 24 samples. However, both SFE and PLE demand a high equipment cost and intensive method development, but result in fairly clean extracts (Dean & Xiong, 2000; Rodil & Moeder, 2008b).

In MAE (Figure 7), the sample and organic solvent are subjected to microwave radiation, either in a sealed (pressurized MAE) or in an open vessel (atmospheric MAE). The partitioning of the analytes of interest from the sample matrix to the extraction solvent depends on the temperature and the nature of the solvent (Camel, 2000). Its main advantage is the possibility of extracting several samples at once, which significantly reduces extraction time. In addition, compared to other extraction techniques, MAE offers high efficiency, improved recovery and repeatability, low solvent consumption, short extraction times, ease of operation and only moderate equipment investment (Antonić & Heath, 2007; Camel, 2000; Dean & Xiong, 2000; R. Liu, Zhou, & Wilding, 2004; Vazquez, Carro, Lorenzo, & Cela, 1997). The major limitation of MAE is the need for additional clean-up (e.g. filtration or centrifugation), required after extraction to remove the sample matrix from the analyte-containing solvent (Dean & Xiong, 2000). Optimization of MAE conditions involves several parameters, such as choice of solvent, reaction time, temperature and pressure, as well as microwave power. The extraction solvent is one of the most important parameters to choose, since it needs to be capable of absorbing microwave energy, and converting energy into heat. This depends on the solvent polarity, dielectric constant and many other characteristics, which are crucial in the selection of extraction solvent or solvent mixture (Camel, 2000; Lambropoulou, 2010). In addition, the solvent polarity should be similar to that of the analyzed compounds (R. Liu et al., 2004). Extraction efficiency also largely depends on the reaction temperature. Elevated temperatures enhance extraction by increasing the diffusivity of the solvent into the internal parts of the matrix, and by enhancing the desorption of the components from the active sites of the matrix. However, the temperature needs to be carefully controlled to avoid degradation of the extracted compounds at higher temperatures (Camel, 2000).

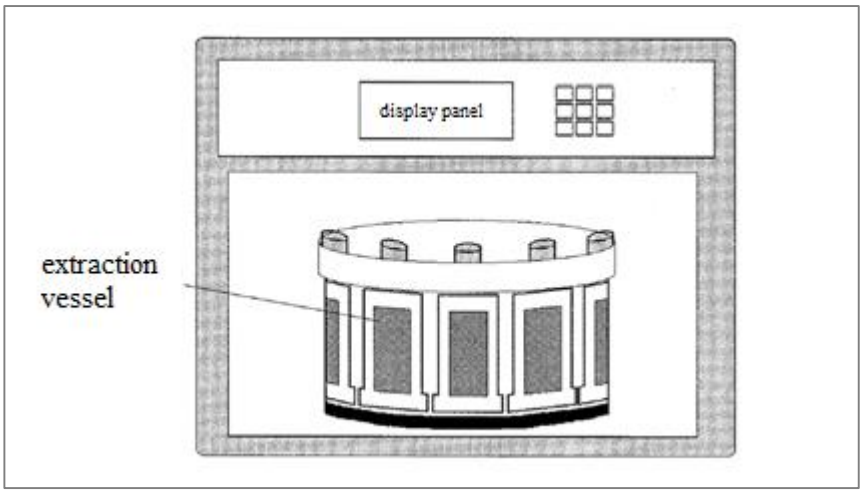


Figure 7: Schematic presentation of a closed vessel MAE system (adapted from Dean & Xiong, 2000).

Extraction of benzophenone derivatives from sediment samples has been mainly performed by using UAE (Kameda et al., 2011; Löffler & Ternes, 2003; Sánchez-Brunete et al., 2011), PLE (Rodil & Moeder, 2008b) and mechanical shaking (Jeon et al., 2006). Only one study, determining ketoprofen in sediments, involved microwave assisted extraction (Antonić & Heath, 2007). Extraction techniques and efficiencies achieved in benzophenones analysis are summarized in Table 10. Extraction efficiencies of all four techniques are comparable; except for mechanical shaking (60–125%) all other methods have efficiencies of ≥ 82%.

Table 10: Extraction methods, applied to the analysis of solid samples for detection of the benzophenone derivatives.

Extraction Technique	Analyte	Extraction Efficiency (%)	Reference
UAE	KP, H-BP, HM-BP, DH-BP, DHM-BP	90–126	(Kameda et al., 2011; Löffler & Ternes, 2003; Sánchez-Brunete et al., 2011)
PLE	HM-BP	88–98	(Rodil & Moeder, 2008b)
MAE	KP	82	(Antonić & Heath, 2007)
Mechanical shaking	BP, H-BP, HM-BP, DH-BP, DHM-BP	60–125	(Jeon et al., 2006)

1.3.3 Derivatization

For gas chromatography, high volatility and thermal stability of the analytes are necessary. Since many organic substances, including most PPCPs, have a relatively low volatility, derivatization is required prior to analysis by GC, resulting in an increased response, which is especially important when determining trace levels of compounds in environmental samples (McArdell et al., 2006).

The choice of a derivatization agent depends on the functional groups of the analytes. Compounds that have –OH, –SH or –NH groups (alcohols, phenols, carboxylic acids, thiols, amines, sulphonamides, etc.) can be derivatized using several different derivatization methods, such as silylation, acylation and alkylation. The most widely used derivatization

method is silylation, where an alkylsilyl group is attached onto the functional moiety of the analyte (McArdell et al., 2006). Alkylsilyl group displaces the reactive hydrogen in -OH, -SH or -NH groups of the analyte, converting it to either silyl ethers or esters. The character of the analyte molecule is changed from polar and active to non-polar and inert, which results in increased volatility and thermal stability (Blau & King, 1977). The ability of specific functional groups to form silyl derivatives is the following: alcohols > phenols > carboxylic acids > amines. A variety of silylation reagents with different properties such as volatility, reactivity, selectivity and by-product formation are available, e.g. N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetimide (MTBSTFA) (Segura, Ventura, & Jurado, 1998). MTBSTFA derivatives have greater thermal and hydrolytic stability compared to other trimethylsilyl derivatives (Kosjek et al., 2011), however MTBSTFA is known to have a lower reactivity than MSTFA and BSTFA (Farré, Petrovic, & Barceló, 2007). Another commonly used derivatization procedure is acylation, where an acyl group is introduced by substitution of a reactive hydrogen in -OH, -SH or -NH groups of the analyte, forming much more volatile esters (Segura et al., 1998). Acylation can be performed using three main types of derivatization reagents: acyl halides, acid anhydrides (e.g. heptafluorobutyric anhydride (HFBA) and trifluoroacetic anhydride (TFAA)) and reactive acyl derivates (e.g. acylated imidazoles, acylated amides or acylated phenols) (Blau & King, 1977). Derivatization by alkylation reactions consists of replacement of the active hydrogen by an alkyl or an aryl group. Most commonly used alkylation reagents are alkyl halides, low-molecular-mass aliphatic bromides and iodides and benzyl bromides (e.g. 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr)) (Segura et al., 1998).

Derivatization of the analytes containing a carbonyl group can be achieved using O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA), which converts the carbonyl group to an oxime. With most of the ketones and aldehydes, two geometric isomers are formed (*E* and *Z*), except in the case of symmetrical carbonyls, however, most studies report that the ratio of the isomers remained stable (Cancilla & Que Hee, 1992; Nawrocki, Kalkowska, & Dąbrowska, 1996).

The presence of hydroxyl moieties in the chemical structure of the investigated benzophenone derivatives (H-BP, DH-BP, HM-BP, DHM-BP and KP) allows their derivatization by silylation reagents such as MSTFA, MTBSTFA and BSTFA, which all have been reported to successfully derivatize these compounds (Cuderman & Heath, 2007; Jeon et al., 2006; Kosjek et al., 2005; Negreira et al., 2009a; Rodil & Moeder, 2008b; Sánchez-Brunete et al., 2011; Tarazona et al., 2010; Zupanc, 2013; Zupanc et al., 2013) (Table 11). Derivatization of BP, EtBP and AcBP to my knowledge has not been reported, although the analysis of BP by gas chromatography has been reported as a non-derivatized compound (Jeon et al., 2006; Migaku Kawaguchi et al., 2006).

Table 11: Derivatization agents used to derivatize benzophenone derivatives.

Derivatization agent	Analyte	Reference
MSTFA	KP, BP, H-BP, HM-BP, DH-BP, DHM-BP	(Antonić & Heath, 2007; Cuderman & Heath, 2007; Jeon et al., 2006; Kosjek et al., 2005; Negreira et al., 2009a)
MTBSTFA	KP	(Zupanc et al., 2013)
BSTFA	H-BP, HM-BP, DH-BP, DHM-BP	(Sánchez-Brunete et al., 2011; Tarazona et al., 2010)

1.3.4 Instrumental analysis

Instrumentation in chemical analysis of organic compounds in environmental samples usually involves two main units: a chromatograph, which separates the analytes, and a detector. In the analysis of PPCPs, separation is typically achieved using either gas (GC) or liquid chromatography (LC), both coupled to a mass spectrometer (MS) (Skoog, Holler, & Nieman, 1998).

1.3.4.1 Separation

Chromatographic techniques are an essential tool in analytical chemistry for separation of a complex mixture, where the sample is transported by a gas or liquid mobile phase through a stationary phase (Skoog et al., 1998). While travelling, compounds interact with specific functional groups on the stationary phase. Each component of the mixture has a different affinity towards the stationary phase, which results in partitioning between the mobile and stationary phase, allowing the separation and qualitative and/or quantitative analysis (McArdell et al., 2006). Based upon the type of mobile phase, gas and liquid chromatographs are used in the environmental analysis of PPCPs.

In gas chromatography, analytes are distributed between the stationary phase and the mobile phase, which is an inert carrier gas (e.g. argon, helium, nitrogen or hydrogen). The sample is injected in a temperature-controlled injection port connected to the chromatographic column, vaporized and transported through the column along with the mobile phase. Each component of the sample reaches the detector at a different retention time, depending on its affinity to the stationary phase (Skoog et al., 1998). Chromatographic parameters such as temperature, gas flow, type and the thickness of stationary phase, column length and width can be adjusted to achieve optimum separation (McArdell et al., 2006). Typical column lengths are between 10 m and 100 m. The most widely used capillary columns are polyimide-coated fused silica columns, which offer several important advantages such as physical strength, low reactivity toward sample components, and flexibility. Although higher resolution columns are available with diameters of 0.20 mm and 0.15 mm, the most widely used silica capillary columns have internal diameters of 0.25 mm and 0.32 mm, which produce 4.630 and 3.760 theoretical plates per meter, respectively (Skoog et al., 1998). Choosing a stationary phase is the most important step in selecting a column. A stationary phase is the film coated on the inner wall of a capillary column, and should be selected based on the physico-chemical properties of the target analytes and their interactions with the stationary phase. The most widely used stationary phases for both packed and capillary columns are as follows (Skoog et al., 1998):

- 5% phenyl-polydimethyl siloxane
- 5% diphenyl-dimethylpolysiloxane
- 5% phenylarylene-dimethylpolysiloxane
- 35% phenyl-dimethyl siloxane
- 50% phenyl-polydimethyl siloxane
- 50% trifluoropropyl-polydimethyl siloxane
- 50% cyanopropyl-polydimethyl siloxane

Gas chromatography analysis is limited to volatile compounds that can be transferred directly into the gas phase (Buchberger, 2011). Therefore, GC is an appropriate method for non-polar, thermostable and volatile substances, while nonvolatile compounds require derivatization (Klein, O'Connor, & Aga, 2008). Although this can be time consuming, the ability to derivatize many different functional groups allows GC to be widely applied in environmental analysis as in the case of PPCPs. When GC is hyphenated to different

detectors, such as MS, it offers high selectivity and sensitivity (Buchberger, 2007).

In liquid chromatography, the sample components are separated due to the differences in how they partition between the liquid mobile phase and solid stationary phase. High-performance liquid chromatography (HPLC) is a commonly applied LC technique in PPCPs analysis, which typically utilizes different types of stationary phases – normal and reversed phase. Normal phase HPLC is characterized by a very polar stationary phase (often consisting of silica gel) and a relatively non-polar mobile phase. For reversed phase HPLC relatively non-polar stationary phase (such as silica gel modified with alkyl groups e.g. octyl (RP-C_8) or octadecyl (RP-C_{18})) and a polar mobile phase are applied. For optimization of the required separation the composition of the mobile phase may be altered (McArdell et al., 2006; Skoog et al., 1998). In recent years, there is a growing trend towards the use of ultra-performance liquid chromatography (UPLC), where the columns are packed with particles of smaller diameter (typically 1.7 μm), allowing higher pressures and higher flow rates. This results in narrower peaks (5–10 s wide) and improved chromatographic separation and sensitivity, shortened analysis time as well as reduced costs in terms of time and solvent consumption (Nováková, Matysová, & Solich, 2006; Richardson & Ternes, 2011). LC is a preferable method in the analysis of polar, non-volatile, and thermally unstable PPCPs. Therefore, it is also suitable for multiclass determination of PPCPs, which is nowadays extremely important in environmental analysis. However, the main drawback of LC is its susceptibility to matrix effects, which affects the methods sensitivity, precision and accuracy (Kosjek, 2009; McArdell et al., 2006; Skoog et al., 1998).

Most benzophenone-derived PPCPs in the environmental samples are measured with GC-based techniques (Table 12), using silica fused capillary columns with 5% diphenyl – 95% dimethylpolysiloxane (Balmer et al., 2005; Cuderman & Heath, 2007; Jeon et al., 2006; Kosjek et al., 2005; Liu et al., 2010; Negreira et al., 2009a; Poiger et al., 2004; Rodil & Moeder, 2008a, 2008b; Sánchez-Brunete et al., 2011; Tarazona et al., 2010; Zenker et al., 2008), 5% phenylarylene – 95% dimethylpolysiloxane (Giokas et al., 2004; Lambropoulou et al., 2002) and arylene stabilized phase equivalent to 35% phenyl – 65% dimethyl siloxane (Kameda et al., 2011) (Table 12). These columns offer high operating temperatures, low bleed, high inertness, selectivity and sensitivity. A few studies also report the use of LC, where mostly reversed phase columns containing silica modified with octadecyl groups (RP-C_{18}) were employed (Farré et al., 2001; Fent, Zenker, et al., 2010; Giokas et al., 2004; Negreira et al., 2009b; Rodil et al., 2008; Vieno et al., 2005; Zenker et al., 2008).

Table 12: Separation techniques applied in the analysis of benzophenones.

	Stationary phase	Analyte	Reference
GC	5% diphenyl – 95% dimethylpolysiloxane	KP, BP, H-BP, HM-BP, DH-BP, DHM-BP	(Balmer et al., 2005; Cuderman & Heath, 2007; Jeon et al., 2006; Kosjek et al., 2005; Liu et al., 2010; Negreira et al., 2009a; Poiger et al., 2004; Rodil & Moeder, 2008a, 2008b; Sánchez-Brunete et al., 2011; Tarazona et al., 2010; Zenker et al., 2008)
	5% phenylarylene – 95% dimethylpolysiloxane	HM-BP	(Giokas et al., 2004; Lambropoulou et al., 2002)
	arylene stabilized phase equivalent to 35% phenyl – 65% dimethyl siloxane	BP, HM-BP	(Kameda et al., 2011)
LC	Silica gels modified with octadecyl groups (RP-C_{18})	KP, HM-BP, DH-BP	(Farré et al., 2001; Fent, Zenker, et al., 2010; Giokas et al., 2004; Negreira et al., 2009b; Rodil et al., 2008; Vieno et al., 2005; Zenker et al., 2008)

1.3.4.2 Detection

Among various detectors that can be coupled to either a GC or LC, the mass spectrometer is considered a principal tool for detection and identification of trace amounts of PPCPs in complex environmental samples (Buchberger, 2011; Kosjek et al., 2011). In addition, MS provides structural characterization of investigated compounds in target and non-target analysis. The overall trends in MS include the increasing use of high resolution (HRMS) and tandem MS (Pestotnik et al., 2014). MS-based detection offers the required sensitivity and selectivity, and provides information about the elemental composition of analytes, and isotopic ratios of atoms (Buchberger, 2011; McArdell et al., 2006; Skoog et al., 1998). The MS instrument consists of an ion source, a mass analyzer and an ion detector. After GC or LC separation, the ion source converts the compounds eluting from the column into either ionized molecules or their fragments, which are then separated according to their mass-to-charge ratio (*m/z*). Finally, a transducer converts the beam of ions into an electrical signal that can be processed and displayed (McArdell et al., 2006; Skoog et al., 1998).

As a source of ions in GC, electron impact (EI) and chemical ionization (CI) are mainly used (Buchberger, 2011; Skoog et al., 1998). EI allows reliable quantification, often identification and good repeatability, which are important in trace analysis of organic compounds; however, CI offers information regarding the molecular ion (McArdell et al., 2006). In LC-MS, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) both provide high sensitivity, however, the sensitivity in the ESI mode is approximately 10-fold higher than in the APCI mode. In addition, the advantage of ESI is that matrix effects can be reduced by decreasing the flow directed into the ion source, which decreases the droplet size and the number of molecules ionized in a given time (Kosjek, 2009). The ionization modes applied in LC-MS, such as ESI and APCI, can be applied in the environmental analysis of vulnerable analytes, where thermal decomposition is avoided (Fenn, Mann, Meng, Wong, & Whitehouse, 1989).

Several mass analyzers are utilized in environmental analysis of emerging contaminants,

which differ in how ions are separated, resulting in different sensitivity, selectivity and capability of ion fragmentation. Despite the existence of newer and improved detection techniques, quantitative analysis of PPCPs is still widely performed using a single quadrupole (Q) MS. Table 13 shows how this applies also to benzophenone derivatives (Antonić & Heath, 2007; Balmer et al., 2005; Cuderman & Heath, 2007; Jeon et al., 2006; Kameda et al., 2011; Kawaguchi et al., 2008; Liu et al., 2010; Poiger et al., 2004; Rodil & Moeder, 2008a; Sánchez-Brunete et al., 2011; Tarazona et al., 2010), where the reported LODs were as low as 5 pg L^{-1} (Fent, Zenker, et al., 2010). A quadrupole mass analyzer consists of four parallel rods, where ions, depending on their *m/z* ratio, either collide with rods or pass unhindered towards the mass detector due to the applied voltages on each set of rods (McArdell et al., 2006). Due to its ease of handling, good quantitative analysis performance and resolution, low maintenance requirements and cost, it remains an important alternative to newer and improved instruments. Nevertheless, more sophisticated MS analyzers are nowadays considered state-of-the-art, allowing high resolution and/or tandem MS detection, such as triple quadrupole (QqQ), time-of-flight (TOF) and ion trap (IT) instruments (Richardson & Ternes, 2011). QqQ offers improved selectivity and sensitivity allowing tandem MS/MS operation, where the first quadrupole preselects the ion of interest, which is then activated in the second quadrupole and finally analyzed in the third quadrupole. QqQ hyphenated to LC is frequently used for quantitative analysis of benzophenones with the reported LODs of 1.5–7 ng L^{-1} (Negreira et al., 2009b; Rodil et al., 2008; Vieno et al., 2005). It also allows limited structural characterization, however it is not suitable for complete structural elucidation of unknown TPs (McArdell et al., 2006). This can be achieved by the use of an ion trap, which presents a 3D version of the quadrupole mass analyzer, where the ions are stored in an ion trap device consisting of two endcap electrodes and one ring electrode between them. The ions are then ejected towards the detector by changing the applied potential, resulting in higher sensitivity compared to Q and the ability to perform multiple MS^n experiments. The MS^n spectra provide important information regarding fragmentation patterns of unknown analytes, which makes the instrument a useful tool for structural elucidation of unknown compounds such as TPs (McArdell et al., 2006). For this reason, GC hyphenated to an IT was applied not only in quantitative analysis of benzophenones (Ho & Ding, 2012; Negreira et al., 2009a), but also for the identification of TPs formed by chlorination of HM-BP (Negreira et al., 2008) (Table 13).

The detection and identification of TPs require the application of sophisticated instrumentation, capable of MS/MS or multiple MS^n experiments as well as HRMS analysis. This is often achieved by using hybrid mass spectrometers, which are constructed by coupling two different types of mass analyzers, enabling sufficient data for unequivocal structural identification of unknown compounds, for example, a combination of Q and TOF (QqTOF), and a combination of Q and linear ion trap (QqLIT) (Buchberger, 2011; Richardson & Ternes, 2011). QqTOF mass detector is a powerful identification tool, providing both high resolution mass measurements and MS/MS fragmentation to resolve the structures of newly-formed compounds, but is not applied to the quantitative / target analysis, since far more capable mass detectors exist in terms of sensitivity, quantitative accuracy and dynamic range (Pestotnik et al., 2014). For example, QqLIT-MS/MS was used to quantitatively determine HM-BP and its known metabolites H-BP, DH-BP and DHM-BP (target analysis), whereas QqTOF-MS/MS was applied in the structural characterization of unknown TPs (non-target analysis), formed during fungal treatment of HM-BP (Gago-Ferrero et al., 2012) (Table 13). An alternative to the QqTOF is the more advanced LTQ Orbitrap that combines a linear ion trap with the Orbitrap analyzer, which uses a Fourier-transform MS to attain a high-resolution mass performance (Pestotnik et al., 2014). The instrument has become very important in the field of untargeted environmental

analysis due to not only its high resolution and high mass accuracy, but also for its wider dynamic range, when compared to QqTOF (Kosjek, Heath, Petrović, & Barceló, 2007; Pestotnik et al., 2014). Despite its important advantages, it is limited by its cost. Nevertheless, the trend in environmental analysis and especially identification of unknown TPs includes the use of novel high-resolution MS instruments such as LTQ Orbitrap. An example of its use is the study where photocatalytic decomposition of the UV filter 4-methylbenzylidene camphor was followed and its TPs were characterized based on their chromatographic behavior, HRMS data and their MS^n fragmentation (Sakkas et al., 2009).

Table 13: Analytical techniques employed in the analysis of benzophenones in environmental samples.

Separation	Detection	Analyte	LOD (ng L^{-1})	Reference
GC	Q MS; EI	KP, BP, H-BP, HM-BP, DH-BP, DHM-BP	0.005–120	(Antonić & Heath, 2007; Balmer et al., 2005; Cuderman & Heath, 2007; Fent, Zenker, et al., 2010; Jeon et al., 2006; Kameda et al., 2011; Kawaguchi et al., 2008; Liu et al., 2010; Poiger et al., 2004; Rodil & Moeder, 2008a; Sánchez-Brunete et al., 2011; Tarazona et al., 2010)
	IT MS; EI	HM-BP, DH-BP, DHM-BP	0.1–3	(Ho & Ding, 2012; Negreira et al., 2009a)
		TPs formed by chlorination of HM-BP	2.4	(Negreira et al., 2008)
LC	QqQ MS; ESI(+), ESI(-)	KP, HM-BP, DH-BP, DHM-BP	1.5–7	(Negreira et al., 2009b; Rodil et al., 2008; Vieno et al., 2005)
	QqLIT MS; ESI(+)	HM-BP, H-BP, DH-BP, DHM-BP	n.a.	(Gago-Ferrero et al., 2012)
	QqTOF MS; ESI(+), ESI(-)	TPs formed during fungal treatment of HM-BP	n.a.	(Gago-Ferrero et al., 2012)

1.3.5 Analytical quality assurance

Analytical quality assurance comprises of a set of measures a laboratory must undertake in order to continuously achieve high-quality data and therefore reliable analytical

measurements (Taverniers, De Loose, & Van Bockstaele, 2004). Since environmental analysis deals with complex matrices and low analyte concentrations, a thorough laboratory quality assurance program is of high importance (McArdell et al., 2006).

The first step of quality assurance in the laboratory represents analytical method validation. The use of validated methods is necessary for analytical laboratories to show their qualifications and competency. The validation plan (the extent of validation and the performance parameters to be evaluated) is determined by the analytical requirements, the study purpose or as defined in regulations (Taverniers et al., 2004). The most important validation parameters involve recovery, linearity, accuracy (precision and trueness), specificity, selectivity, sensitivity, limit of detection (LOD), limit of quantification (LOQ), and robustness (Taverniers et al., 2004), which are explained as follows:

- Recovery involves the efficiency of the analytical method in detecting all of the analyte present in a sample. It is defined as the fraction of the analyte added to the test sample prior to analysis, measured by the analytical method. Linearity is the ability of the analytical method to obtain test results proportional to the concentration of the analyte within a given range.
- Linear range (working range) is the range of concentrations or amounts of analyte over which the method gives test results proportional to the concentration of the analyte and is usually represented as a calibration curve.
- Accuracy is defined as the closeness of agreement between a test result and the accepted reference method. It is expressed by precision and trueness.
- Precision is expressed as the closeness of the agreement between independent test results and is reported as standard deviation (SD) or relative standard deviation (RSD). Precision measures are divided into repeatability precision measures (when the same analyst uses the same equipment in the same laboratory in short intervals), intra-laboratory (when different analysts use the same equipment over extended period) and inter-laboratory reproducibility (when different laboratories with different operators using different equipment perform the analysis).
- Trueness is expressed as the closeness of the agreement between the mean value determined for the analyte of interest and the accepted true value. To determine trueness, reference materials are used, which are divided into certified reference materials (CRMs) and non-certified (laboratory) materials. CRMs are defined by a statement of uncertainty and are traceable to international standards. Therefore, they can be used to validate methods, for comparison of the methods and for calibration of equipment and methods. When no CRMs are available, a blank sample matrix can be spiked with a known amount of a pure stable in-house material/standard.
- Specificity is the ability of the method to determine accurately and specifically the analyte of interest in the presence of other interfering components in a sample matrix. Specificity is defined as 100% selectivity.
- Sensitivity refers to the change in the response of a measuring instrument divided by the corresponding change in the stimulus.
- Limit of detection (LOD) is the lowest amount or concentration of an analyte in a sample, which can be detected but not necessarily quantified as an exact value. It is expressed as the concentration or a quantity, derived from the smallest signal, which can be detected with reasonable certainty for a given analytical procedure.
- Limit of quantification (LOQ) is the lowest amount or concentration of an analyte that can be determined quantitatively with an acceptable level of repeatability, precision and trueness.
- Robustness is a measure of the capacity of the analytical procedure to remain unaffected by small, but deliberate variations in method-performance parameters,

which provides an indication of its reliability during normal usage (Taverniers et al., 2004).

Further, to ensure quality of results in the analytical procedure blank controls, internal and instrumental standards can be applied. Internal standard is a substance with similar physico-chemical properties as the investigated analyte. It is added to the sample in the first step of analytical method (for example prior to sample preparation) as it compensates for losses of analytes during sample preparation and detection. On the contrary, instrumental standard is added just before the introduction of a sample into analytical instrument, i.e. prior to injection onto the column. It accounts for the correctness and reliability of an analytical instrument. In the analysis of PPCPs, ^{2}H (deuterium), ^{13}C or ^{15}N isotopically labelled analogues of analytes are mostly used as internal standards. If the isotopic analogues are not available, the use of other structurally similar compounds can be applied (Buchberger, 2011). By using blank controls, possible contamination is detected and false positive results can be avoided. Procedural blanks control the laboratory contamination during sample preparation, while instrumental blanks control the performance of instruments during a series of measurements (McArdell et al. 2006).

2 Aims and Hypothesis

Each day, numerous emerging contaminants enter the aquatic environment, but their presence in natural waters remains unregulated. Due to increasing consumption and possible toxic effects of benzophenone derivatives on aquatic ecosystems, it is crucial to investigate their presence in different environmental compartments and to understand their environmental fate and effects. Nevertheless, these data are scarce and insufficient to provide a reliable environmental risk assessment. There is also a lack of analytical procedures for their trace level determination in environmental samples, especially in sediments. In addition, reports concerning the environmental fate of these compounds under actual environmental conditions are missing, which would provide a better understanding of their cycling in the environment. Furthermore, the data regarding the toxicity of benzophenone derivatives and their transformation products is incomplete and deserves special attention.

The main objectives of this thesis were to:

- develop, optimize and validate an analytical method for the simultaneous determination of eight benzophenone derivatives in surface waters and sediments
- quantify selected benzophenones in the aqueous environment – rivers, lakes, sea, and corresponding sediments
- investigate the photostability and determine the degradation kinetics of the selected benzophenone derivatives under the influence of artificial UV light and natural sunlight
- evaluate the genotoxicity of the parent benzophenones and their photodegradation mixtures, produced by UV and solar irradiation

To achieve these aims, the following hypotheses were tested:

- H1: Microwave assisted extraction yields higher extraction efficiencies for the investigated benzophenones from sediments than ultrasonic extraction.
- H2: Benzophenone-based compounds are present in Slovenian environment.
- H3: The type of aqueous matrix influences the photolytic behavior of selected benzophenones.
- H4: UV laboratory experiments can serve as the basis for predicting the environmental fate of selected benzophenones under the influence of solar irradiation.
- H5: Benzophenone derivatives have genotoxic potential.

3 Materials and Methods

3.1 Analytical Method for the Determination of Benzophenones in Aqueous and Solid Matrices

3.1.1 Standards

Analytical standards of benzophenone derivatives BP (CAS:119-61-9), H-BP (CAS: 1137-42-4), DH-BP (CAS: 131-56-6), DHM-BP (CAS: 131-53-3) and KP (CAS: 22071-15-4) were purchased from Sigma – Aldrich (Steinheim, Germany), while HM-BP (CAS: 131-57-7) was obtained from Merck (Damnstadt, Germany). All were of high purity (> 97%). AcBP (CAS: 66067-44-5) was supplied by Synchem OHG (Felsberg-Altenburg, Germany), while EtBP (CAS: 66067-43-4) was custom synthesized by using Friedel-Crafts alkylation at the Faculty of Chemistry and Chemical Technology, University of Ljubljana (Ljubljana, Slovenia) (Prebil, 2010).

To prepare stock solutions, an adequate amount of the benzophenone standard was accurately weighed out using an analytical balance (Mettler Toledo AT201; Greifensee, Switzerland) and diluted with either methanol (MeOH) or acetonitrile (ACN). The solutions prepared in MeOH were used for method development and the determination of benzophenones in environmental samples, while ACN was used for photodegradation experiments to prevent radical scavenging. Different working standards were prepared by diluting these stock solutions. All standard solutions were stored in the dark at 4 °C.

3.1.1.1 Internal standards

Four different internal standards were used in the experiments: benzophenone-2,3,4,5,6-d_5 (BP-d_5), 2-hydroxy-4-methoxybenzophenone-2',3',4',5',6'-d_5 (HM-BP-d_5), ketoprofen-d_3 (α-methyl-d_3) (KP-d_3) and 3-i-propylbenzophenone (iPrBP). Table 14 shows the internal standards with their corresponding analytes.

Table 14: Benzophenone derivatives with their corresponding internal standards.

Analyte	Internal standard
BP	BP-d_5
H-BP	HM-BP-d_5
DH-BP	HM-BP-d_5
HM-BP	HM-BP-d_5
DHM-BP	HM-BP-d_5
KP	KP-d_3
EtBP	iPrBP
AcBP	iPrBP

The isotopically labelled internal standards BP-d_5, HM-BP-d_5 and KP-d_3 were provided by CDN Isotopes (Quebec, Canada), while iPrBP were custom synthesized using Friedel-Crafts alkylation at the Faculty of Chemistry and Chemical Technology, University of

Ljubljana (Prebil, 2010).

3.1.2 Chemicals

The derivatization reagent N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetimide (MTBSTFA) was obtained from Acros Organics (Fair Lawn, NJ, USA), while N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) were purchased from Sigma – Aldrich (Steinheim, Germany). The derivatization reagent PFBHA was dissolved in methanol to give a concentration of 6 g L^{-1} (PFBHA solution).

The solvents used: ethyl acetate (EtOAc), methanol (MeOH), acetonitrile (ACN), dichloromethane (DCM), acetone (ACT) and hexane (HEX) were obtained from J.T. Baker (Deventer, the Netherlands), hydrochloric acid (37%) was supplied by AppliChem (Darmstadt, Germany) and formic acid (≥ 98%) by Sigma – Aldrich (Steinheim, Germany). All were of analytical grade purity.

3.1.3 Sample collection and handling

Water and sediment samples were collected by grab sampling during July – September 2013 from locations in Slovenia and Croatia. Due to a variety of possible pollution sources, the selected sample sites represent either areas with a high intensity of water related recreational activity (5 lakes, 3 rivers and 3 sea sites) or receiving water bodies of WWTPs (3 rivers). Five lakes (Lake Bled, Lake Bohinj, Lake Šobec, Lake Rakitna, and Lake Bloke), three rivers (Krka, Nadiža, and Kolpa) and three sites on the Northern Adriatic Sea (Portorož, Ankaran, and Novigrad) were sampled in the areas of recreational activity at peak recreational times. The rivers Ljubljanica, Krka, and Kamniška Bistrica were sampled near the WWTP outflow, downstream of the plant. Details of the sampling locations are presented in Figure 8 and Table 15.

Water samples were collected from coastal sites using grab sampling, stored in glass containers and immediately transported at 4 °C to the laboratory. Each sample was filtered through a 0.45 μm cellulose nitrate membrane filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany) and stored in the dark at -20 °C prior to analysis.

Sediment samples were collected from the top 10 cm sediment layer of the shoreline areas of lakes, rivers and the sea using a stainless steel scoop (grab sampler). They were then stored in amber glass containers at 4 °C, while being transferred to the laboratory, where they were dried at 40 °C to a constant weight in the dark. Once dried, the sediments were sieved to give two fractions, one with particle sizes < 2 mm used for texture determination and the second with a particle size of < 200 μm for sediment analysis. Sieved sediments were stored at -20 °C.

For method development and validation, water samples taken from the Drava River, Lake Podpeč and seawater from Novigrad served as blank samples, while the sediment blank was collected from a stream in Mostec, Ljubljana, Slovenia. These samples were collected in spring, before the peak season and their analysis revealed the presence the target compound < LOD.

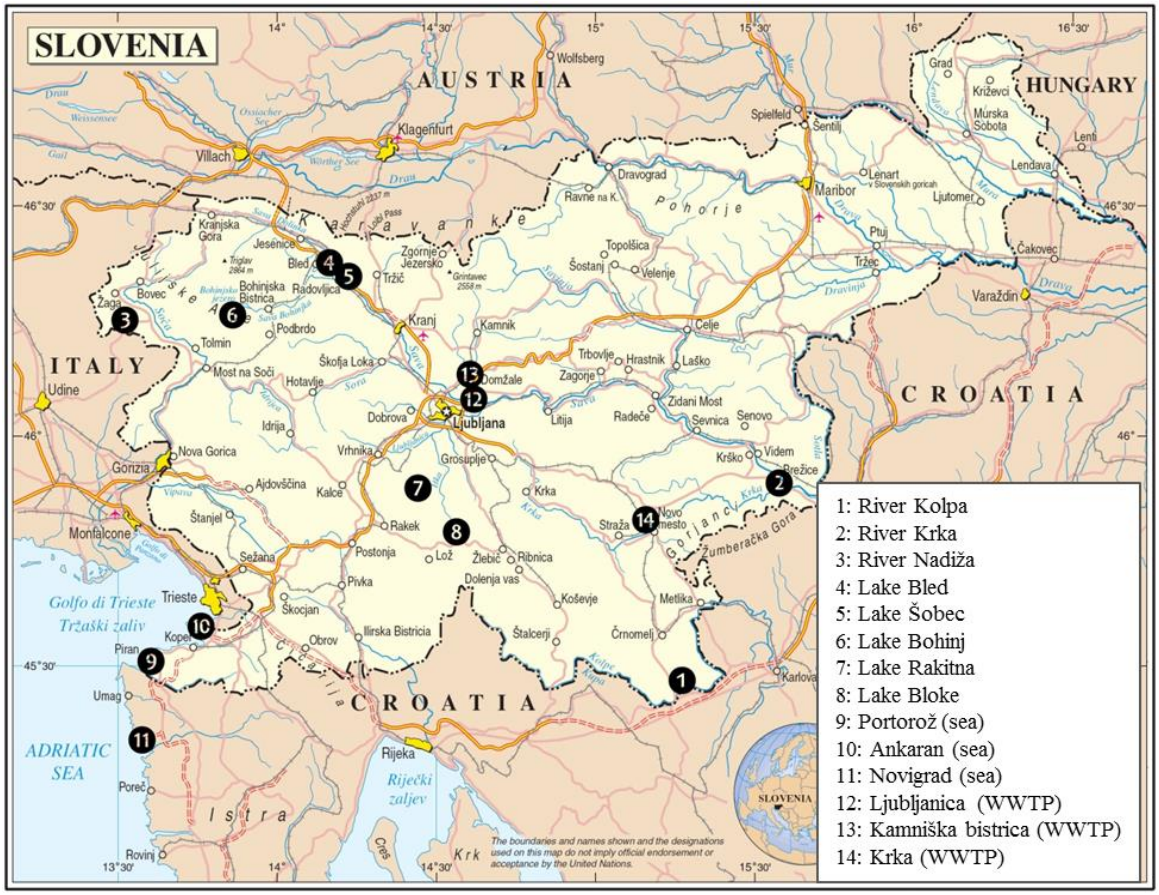


Figure 8: Sampling sites in Slovenia and Croatia (map adapted from http://www.un.org/Depts/Cartographic/map/profile/slovenia.pdf).

Table 15: Geographic coordinates of the sampling locations.

Matrix	Sampling location	Latitude (Decimal degrees)	Longitude (Decimal degrees)
River	Kolpa	45.459	15.256
	Krka	45.886	15.564
	Nadiža	46.244	13.507
Lake	Bled	46.362	14.082
	Šobec	46.354	14.151
	Bohinj	46.280	13.839
	Rakitna	45.887	14.432
	Bloke	45.786	14.516
Sea	Portorož	45.512	13.594
	Ankaran	45.578	13.732
	Novigrad	45.323	13.559
River (WWTP)	Ljubljanica	46.066	14.624
	Kamniška Bistrica	46.117	14.611
	Krka	45.818	15.182

3.1.4 Sample preparation

3.1.4.1 Sample preparation for water samples

Aqueous samples were extracted using solid phase extraction (SPE), which was optimized regarding sample volume, pH, elution solvent and matrix effect. The optimal conditions included a procedure, where a total of 800 mL of sample was acidified to pH 2–3 using 37% HCl and spiked with the internal standards (KP-d_3, HM-BP-d_5, BP-d_5 and iPrBP) to give a concentration of 400 ng L^{-1}. Solid phase extraction was performed by using 60 mg / 3 mL Oasis HLB reversed phase sorbent cartridges (Waters Corporation, MA, USA). For each sample, a cartridge was pre-conditioned with 3 mL of EtOAc and 3 mL of MeOH, before being equilibrated with 3 mL of acidified water (pH 2–3). The sample was passed through the SPE cartridge at a flow-rate of approximately 2 mL min^{-1}. The cartridge was vacuum-dried and further eluted with 1800 µL of EtOAc (4 × 450 µL). The eluant was dried under a gentle stream of nitrogen and re-dissolved in 600 µL of EtOAc. Due to different derivatization processes for individual compounds, the extract was divided in two aliquots of 300 µL.

3.1.4.2 Sample preparation for sediment samples

Sediment sample preparation involved microwave assisted extraction (MAE) using the Microwave Accelerated Reaction System (MARS) 5 (CEM corporation, Matthews, NC, USA) in a closed vessel system (GreenChem). To achieve the best performance of MAE, several parameters were tested including reaction temperature, time, power and extraction solvent. Optimized method included adding a known amount of dry sediment (4 g) to an extraction vessel with the addition of 30 mL of MeOH/ACT (1:1, v/v) with 5% (w/w) formic acid. The sample was extracted at 800 W at 150 °C for 30 min (heating ramp to 150 °C for 10 min) and then allowed to cool to room temperature. Samples were centrifuged for 10 min at 3500 rpm (Centrifuge LC-320, Tehtnica, Železniki, Slovenia). The supernatant was transferred into a round-bottom flask, while the remaining sediment was washed with a further 10 mL of MeOH before being centrifuged for a second time. The supernatants were combined and the solvent removed by using rotary evaporation. The dry residue was then diluted with water to 100 mL and cleaned-up on an SPE cartridge following the same procedure as for the water samples.

3.1.4.3 Derivatization

The derivatization process was optimized to achieve good chromatographic properties and high sensitivity for detection of benzophenones by GC-MS. Four factors affecting the derivatization (derivatization agent, reaction time, temperature and solvent) were evaluated. To perform derivatization under the determined optimal conditions, the SPE extracts were divided into two aliquots, each derivatized separately. The choice of derivatizing agent depended on the compound type (Table 16). For this, 30 µL of MSTFA were added to the first aliquot of 300 µL EtOAc and left to derivatize for 1 h at 60 °C. The other aliquot was dried under a gentle flow of nitrogen and re-dissolved in 300 µL of a PFBHA solution. Derivatization was performed at 60 °C, for 1 h for AcBP and further 14 h for BP, EtBP, iPrBP and BP-d5. To avoid decomposition, the samples were analyzed by GC-MS immediately after derivatization.

Table 16: Derivatization reagents and the derivatization conditions used.

Analyte	Derivatization reagent	Reaction time	Reaction temperature
BP	PFBHA	15 h	60 °C
H-BP	MSTFA	1 h	
DH-BP	MSTFA	1 h	
HM-BP	MSTFA	1 h	
DHM-BP	MSTFA	1 h	
KP	MSTFA	1 h	
EtBP	PFBHA	15 h	
AcBP	PFBHA	1 h	
iPrBP	PFBHA	15 h	
BP-d_5	PFBHA	15 h	
HM-BP-d_5	MSTFA	1 h	
KP-d_3	MSTFA	1 h	

3.1.5 Instrumental analysis

Samples were analyzed using an HP 6890 (Hewlett-Packard, Waldbronn, Germany) gas chromatograph fitted with a single quadrupole mass selective detector HP 5972 (Hewlett-Packard, Waldbronn, Germany) (GC-MSD). Separation was achieved using a DB-5 MS 30 m × 0.25 mm × 0.25 µm (Agilent J&W, CA, USA) capillary column with helium as the carrier gas (37 cm s^{-1}). A 1 µL sample was injected in splitless mode at 250 °C. Depending on the derivatization procedure, the subsequent separation on GC was performed using two different temperature programs. For compounds derivatized with MSTFA, the GC oven was programmed as follows: an initial temperature of 100 °C was ramped at 25 °C/min to 200 °C, at 10 °C/min to 250 °C, held for 1.3 min and then post run for 3 min at 300 °C. The total GC run time was 13.30 min. For compounds derivatized with PFBHA, the GC oven was initially set at 100 °C and further ramped at 25 °C/min to 230 °C, at 10 °C/min to 270 °C, at 30 °C/min to 300 °C, and finally held for 1 min. With a post run of 2 min at 300 °C the total GC run time was 15.20 min. The MS was operated in EI ionization mode at 70 eV. Quantitative determination was achieved using selective ion monitoring (SIM) of the following ions: *m/z* **377**, 360, 196 for BP, *m/z* **270**, 255, 193 for H-BP, *m/z* **343**, 358, 271 for DH-BP, *m/z* **285**, 300, 242 for HM-BP, *m/z* **373**, 388, 299 for DHM-BP, *m/z* **282**, 311, 295 for KP, *m/z* **405**, 388, 224 for EtBP, *m/z* **419**, 161, 105 for AcBP, *m/z* **419**, 376 for iPrBP, *m/z* **382**, 365 for BP-d_5, *m/z* **290**, 305 for HM-BP-d_5 and *m/z* **285**, 314 for KP-d_3 (quantification ions are in bold). For instrumental control and data processing Chemstation software was used.

3.1.6 Schematic presentation of the analytical method

The developed method for determining selected benzophenone derivatives in aqueous and sediment samples is presented in Figure 9 and Figure 10.

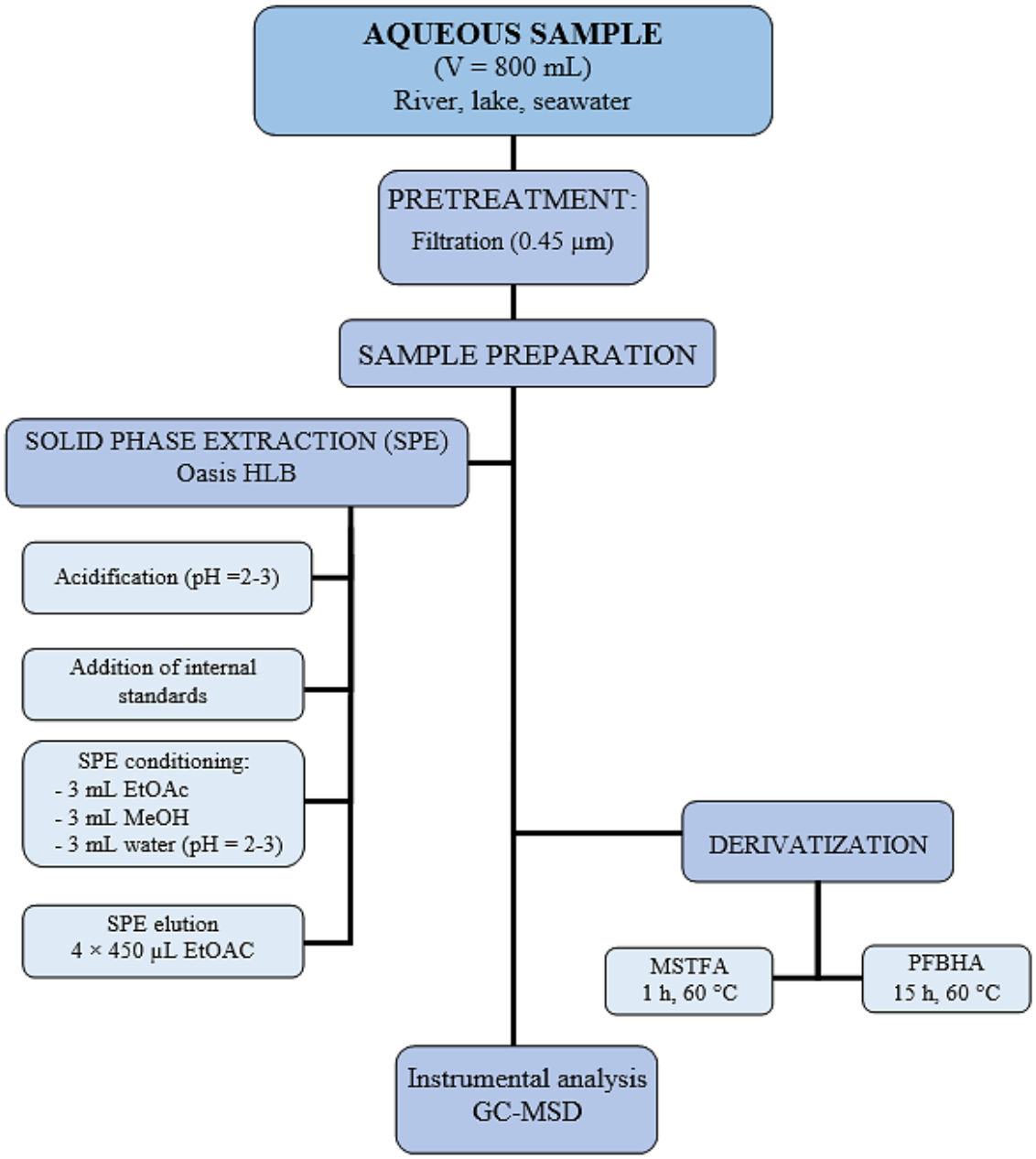


Figure 9: Schematic presentation of the analytical procedure for determination of the target benzophenones in surface waters.

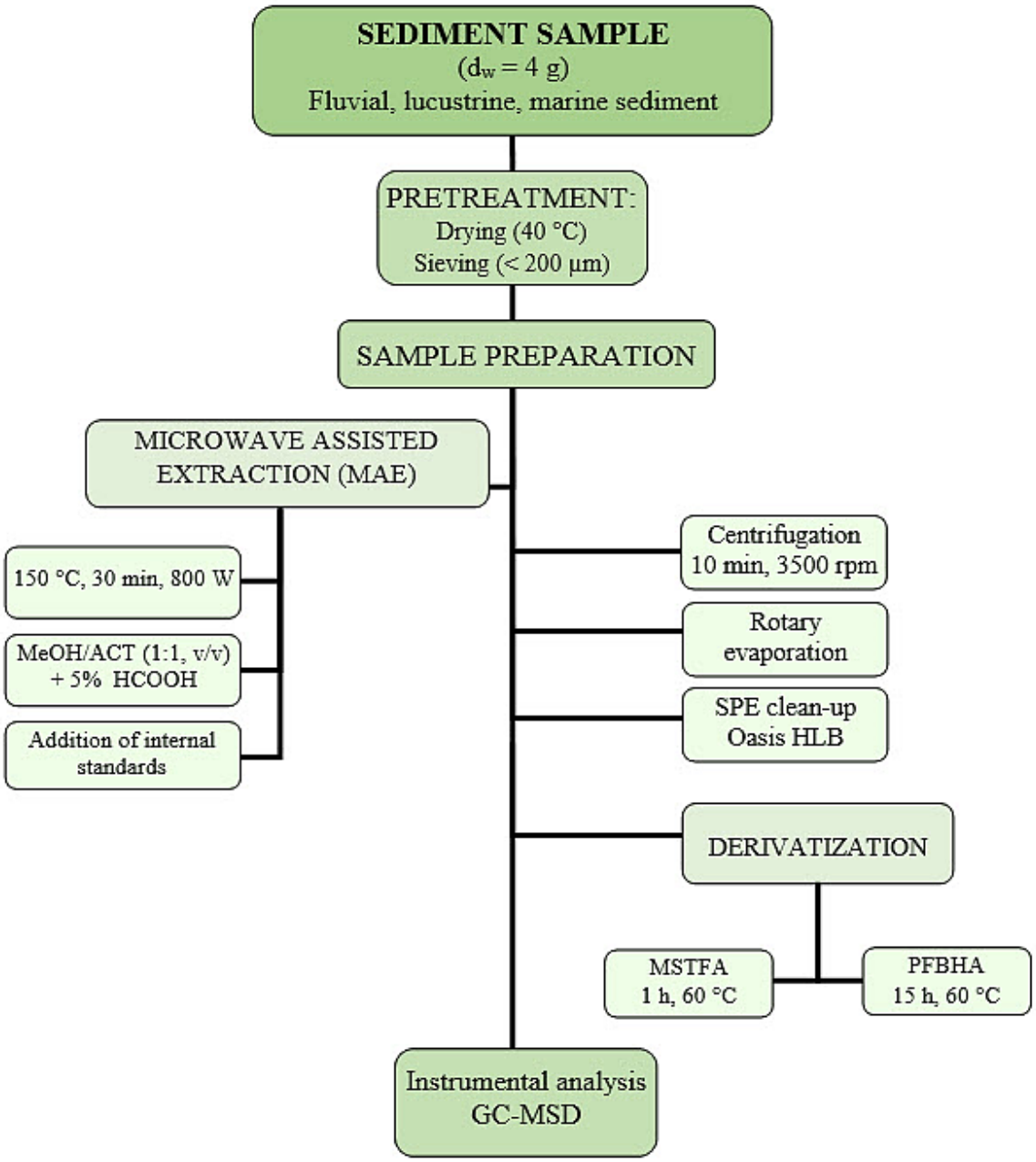


Figure 10: Schematic presentation of the analytical procedure for determination of the target benzophenones in sediment samples.

3.1.7 Analytical method validation

Method performance was evaluated by determining linearity, accuracy, repeatability, sensitivity and extraction efficiency for both water and sediment sample matrices. The linearity of the method was assessed by plotting the ratio of peak area of a target compound to its internal standard *versus* its concentration for each matrix. The r^2 value was determined from the regression curve. Accuracy was expressed as a quotient between the mean observed concentration and the spiked concentration at two concentration levels in three replicates for each matrix. Repeatability was assessed as instrumental (repeatability of measurement) and method repeatability (repeatability of sample preparation and analysis). The instrumental repeatability was determined at two concentrations as the standard deviation of three consecutive injections of the same sample. The method repeatability was determined as the standard deviation of triplicate measurements obtained at two

concentration levels by analysis of the target compounds in spiked water and sediment samples. Sensitivity was expressed as the LOD and LOQ, which were calculated as 3-times (LOD) and 10-times (LOQ) the standard deviation of the baseline for three background samples of surface water and sediment. The SPE efficiency for water samples was calculated as the ratio between the mean peak area of the spiked analyte prior to SPE to that obtained by spiking the same amount of the analyte into the extract post SPE. This was performed at two concentration levels in three replicates and further compared in different aqueous matrices (tap water, river, lake and seawater). MAE efficiency for sediment samples was calculated as a quotient of the mean peak area of the analyte spiked before microwave extraction, with that obtained by spiking the same amount of the analyte into the extract after extraction. Extraction efficiency for sediment samples was determined in triplicate at four different fortification levels.

3.2 Photodegradation Experiments

3.2.1 UV irradiation

Laboratory-scale photodegradation experiments were carried out in a cylindrical glass reactor (Figure 11) by exposing 760 mL of aqueous solution of each individual benzophenone compound in distilled water to UV irradiation. When determining the degradation kinetics of the investigated compounds, the initial concentration used was 1 $\mu g\ L^{-1}$ (containing 0.025% v/v acetonitrile). Acetonitrile was chosen as a co-solvent in order to avoid radical scavenging in the photolysis solution (MacManus-Spencer et al., 2011; OECD, 2008). A medium pressure (MP) mercury lamp and a monochromatic low pressure (LP) mercury UV lamp (Photochemical Reactors Ltd, London, GB) were used as a source of radiation. The LP lamp (6 W) emits 90% of its radiation at 254 nm, while the MP lamp (125 W) has a much more intense arc and radiates predominantly at 365–366 nm with smaller amounts in the ultraviolet region at 265, 297, 303, 313 and 334 nm and significant amounts in the visible region at 404–408, 436, 546 and 577–579 nm. The light intensity, measured by ferrioxalate actinometry (Kete, 2008), was 1.5×10^{-6} Einstein s^{-1} for the MP lamp and 0.78×10^{-6} Einstein s^{-1} for the LP lamp. The temperature of the medium during experiments was maintained between 21–23 °C using a water-cooled immersion well. Homogeneity of the sample was achieved by continuous stirring using a magnetic stirrer (400 rpm).

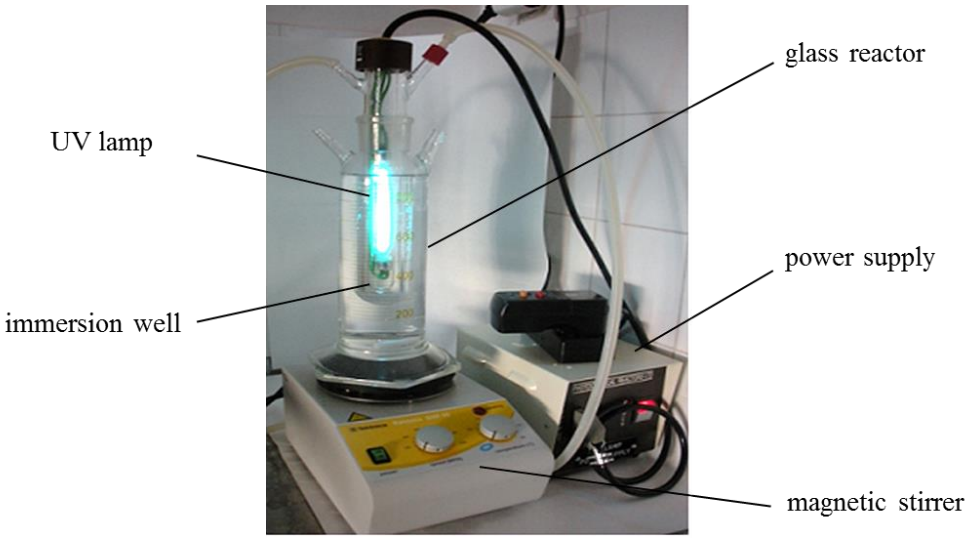


Figure 11: Cylindrical glass reactor used for MP/UV and LP/UV treatment.

To determine the degradation kinetics of benzophenones, including the degradation rate constant (*k*) and half-life ($t_{1/2}$), different exposure times were used. Based on preliminary experiments, irradiation time depended on the elimination efficiency of each test compound, involving three time intervals: 0–10 min (KP), 0–180 min (EtBP) and 0–24 h (BP, H-BP, HM-BP, DH-BP, DHM-BP, AcBP). The elimination efficiency was evaluated as a ratio between treated and non-treated samples (c_t / c_0), expressed as a percentage, where c_t represents the concentration at time *t* and c_0 is the initial concentration.

3.2.2 Solar irradiation

Solar degradation experiments were performed by exposing 1 L of a spiked aqueous solution of each individual benzophenone (1 µg L^{-1}) to sunlight (Figure 12). To evaluate the influence of different aqueous matrices on the photodegradation process, distilled water, seawater (Novigrad, Croatia) and lake water (Lake Podpeč, Jezero, Slovenia) were used. Each matrix was previously analyzed to confirm that none of the test compounds was present in the water sample. Samples in 1 L quartz Erlenmeyer flasks were then irradiated for 4 weeks in Ljubljana, Slovenia (latitude 46.1, longitude 14.5) in August 2013 under clear sky conditions with a daily air temperature of 12 to 36 °C. During this period, there were 15 h of sunshine per day with a maximum of 932 W m^{-2} of local sunlight irradiation intensity (data accessed from http://meteo.arso.gov.si/, daily between 1–31th of August 2013). A control solution of each benzophenone derivative spiked in each of the aqueous matrices tested was covered in aluminium foil and kept under the same conditions to determine any losses due to heat or adsorption to glass. The sampling frequency was chosen based on preliminary studies and times of exposure were the following: 0–5 h (KP), 0–5 days (EtBP) and 0–28 days (BP, H-BP, HM-BP, DH-BP, DHM-BP, AcBP). For each matrix, the degradation kinetics parameters *k* and $t_{1/2}$ were determined.



Figure 12: Solar irradiation experiment set-up.

3.2.3 Chemical analysis of photodegradation mixtures

After irradiation, aqueous samples were immediately analyzed according to the procedure for water samples, described in Section 3.1.4 and 3.1.5. Briefly, 200 mL of water samples were extracted by SPE, extracts were then derivatized by either MSTFA or PFBHA, and analyzed using GC-MS.

3.3 Genotoxicity Testing

3.3.1 Standards

In the SOS/*umuC* assay, the same standards as for chemical analysis were used (Section 3.1.1). Standards of the benzophenone derivatives were prepared in dimethylsulfoxide (DMSO) and then further diluted to give a series of working standards.

3.3.2 Chemicals

Ampicillin, glucose-6-phosphate (disodium salt), D-(+)-glucose (anhydrous), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), sodium dodecyl sulphate (SDS), Na_2CO_3, $MgCl_2 \cdot 6H_2O$ and Na_2HPO_4 and DMSO were obtained from Sigma – Aldrich (St. Louis, USA), nicotinamide adenine dinucleotide phosphate (NADP) (sodium salt), NaCl, $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot H_2O$ from Merck (Darmstadt, Germany), while O-nitrophenyl-ß-D-galactopyranoside (ONPG), ß-mercaptoethanol, KCl and $MgSO_4 \cdot 7H_2O$ were purchased from Fluka (Buchs, Switzerland). Lyophilized Aroclor 1254 induced male rat liver post-mitochondrial fraction (S9) was obtained from Moltox (Boone, USA) and tryptone from BD Bacto (Sparks, USA). All chemical reagents were of the purest grade available.

3.3.3 Preparation of solutions and test medium

Performance of the SOS/*umuC* assay involved several test solutions, which were prepared

as follows:

1x TGA medium	**(1000 mL)**
Trypton	10 g
NaCl	5 g
HEPES	11.9 g
Bidistilled water	980 mL

The components were mixed, dissolved in bidistilled water and calibrated at pH 7.0 ± 0.2. The solution was autoclaved (121 °C, 20 min).

D-(+)-glucose (anhydrous)	2 g
Distilled water	20 mL

After autoclaving, the glucose solution was added to 1x TGA in sterile conditions.

Ampicillin	50 mg

Finally, ampicillin was added to the 1x TGA medium, which was then stored at -20 °C.

10x TGA medium	**(100 mL)**
Trypton	10 g
NaCl	5 g
HEPES	11.9 g
Bidistilled water	80 mL

The components were mixed, dissolved in bidistilled water and calibrated at pH 7.0 ± 0.2. The solution was autoclaved (121 °C, 20 min).

D-(+)-glucose (anhydrous)	2 g
Distilled water	20 mL

After autoclaving, the glucose solution was added to 10x TGA in sterile conditions.

Ampicillin	50 mg

Finally, ampicillin was added to the 10x TGA medium, which was then stored at -20 °C.

10x TGA medium with cofactors	**(100 mL)**
Trypton	10 g
NaCl	5 g
KCl	2.46 g
$MgCl_2 \cdot 6H_2O$	1.63 g
HEPES	11.9 g
Bidistilled water	80 mL

The components were mixed, dissolved in bidistilled water and calibrated at pH 7.0 ± 0.2. The solution was autoclaved (121 °C, 20 min).

D-(+)-glucose (anhydrous)	2 g
Distilled water	20 mL

After autoclaving, the glucose solution was added to 10x TGA in sterile conditions.

Ampicillin	50 mg

Finally, ampicillin was added to the 10x TGA medium, which was then stored at -20 °C.

NADP (sodium salt)	148 mg
glucose-6-phosphate (disodium salt)	76 mg

On the day the test was performed, NADP and glucose-6-phosphate were added to the 5 mL of 10x TGA medium with cofactors.

B-buffer	**(1000 mL)**
$Na_2HPO_4 \cdot 2H_2O$	20.18 g
$NaH_2PO_4 \cdot H_2O$	5.5 g
KCl	0.75 g
$MgSO_4 \cdot 7H_2O$	0.25 g

The components were mixed, dissolved in bidistilled water and calibrated at pH 7.0 ± 0.2.

Sodium dodecyl sulphate (SDS)	1 g

SDS was added to the 1000 mL of the B-buffer.

ß-mercaptoethanol	2.7 mL

Just before the test, ß-mercaptoethanol was added to the B-buffer (1000 mL), which was then stored at 4 °C.

Phosphate buffer	**(100 mL)**
$Na_2HPO_4 \cdot 2H_2O$	1.086 g
$NaH_2PO_4 \cdot H_2O$	0.538 g
Bidistilled water	100 mL

The components were mixed, dissolved in bidistilled water and calibrated at pH 7.0 ± 0.2. The solution was then autoclaved (121 °C, 20 min) and stored at 4 °C until use.

Stop reagent	**(1000 mL)**
Na_2CO_3	105.99 g
Bidistilled water	1000 mL

Na_2CO_3 was dissolved in bidistilled water and stored at 4 °C until use.

ONPG (O-nitrophenyl-ß-D-galactopyranoside) solution	**100 mL**
ONPG	450 mg
Phosphate buffer	100 mL

ONPG was dissolved in phosphate buffer. Due to low solubility of ONPG, the solution was prepared in advance and stored at 4 °C in the dark.

$MgCl_2$-KCl mix	**(500 mL)**
$MgCl_2 \cdot 6H_2O$	40.7 g
KCl	61.5 g
Distilled water	500 mL

$MgCl_2 \cdot 6H_2O$ and KCl were dissolved in distilled water and stored at 4 °C until use.

0.2 M PBS	**(500 mL)**
0.2 M Na_2HPO_4	440 mL
0.2 M $NaH_2PO_4 \cdot H_2O$	100 mL

The solutions were mixed and calibrated at pH 7.4.

10% S9 mix	**(5 mL)**
$MgCl_2$-KCl mix	100 µL
0.2 M glucose-6-phosphate	125 µL
0.2 M NADP	500 µL
0.2 M PBS	2.5 mL

The components were mixed, filtered through 0.22 µm pores, and stored at 4 °C.

S9	500 µL
1x TGA with bacterial culture	1.5 mL

Just before the experiment, S9 and TGA with bacterial culture was added. S9 mix was kept on ice during the whole experiment.

3.3.4 SOS/*umuC* test

The genotoxic potential of the parent benzophenone compounds was tested using the classic SOS/*umuC* assay (Reifferscheid et al., 1991), while genotoxicity of the photodegradation mixtures, produced by MP/UV treatment of the benzophenones, was tested following the SOS/*umuC* protocol for unconcentrated water samples. The method followed the ISO/CD 13829 standard (ISO, 2000) with minor modifications (Žegura et al., 2009).

To perform the SOS/*umuC* test, *Salmonella typhimurium* TA1535 carrying a multicopy plasmid pSK1002 with fused *umuC-lacZ* genes and the gene for resistance to ampicillin was used for the test strain. Due to the lack of microsomal enzymes in the bacterial cells, metabolic activation was applied by adding rat liver post-mitochondrial fracture (S9).

Tests were conducted in triplicate on a microtiter plate, with (+ S9) and without (- S9) metabolic activation. 4-nitroquinoline-N-oxide (NQNO) was used as a positive control without metabolic activation (0.25 µg mL^{-1} and 5 µg mL^{-1}) and aflatoxin B1 (AFB1) with metabolic activation (5 µg mL^{-1}), while distilled water was used as a negative control.

3.3.4.1 Classic SOS/*umuC* test

The bacterial culture was incubated overnight with shaking at 37 °C, then diluted 10-times with fresh 1x TGA medium and again at 37 °C for 1.5 h with shaking until the bacteria

reached the exponential growth phase.

Ten-microliter aliquots of benzophenone derivatives in concentrations 7.81–1000 μg mL^{-1} were transferred onto a microtiter plate. The initial incubation mixture (Plate A) consisted of 10 μL of each compound dissolved in 10% DMSO, and 90 μL of bacterial culture. For metabolic activation, 10 μL of a test compound was incubated with 90 μL of the S9 bacterial culture mixture, containing 10% of S9. To ensure accurate results, blind, solvent, negative and positive controls were used (Table 17).

The microtiter plate was incubated at 37 °C for 2 h with shaking (150 rpm). The incubation mixture was then diluted 10-times with fresh warm 1x TGA medium (Plate B), following a subsequent additional incubation period of 2 h at 37 °C. Bacterial growth rate (G) was determined by measuring absorbance at 600 nm.

The sample (30 μL) was then transferred to another microtiter plate (Plate C), containing 120 μL of B-buffer. Immediately, 30 μL of ONPG was added. After 30 min of shaking at 25 °C using ONPG as a substrate, 120 μL of stop reagent (Na_2CO_3) was added. ß-galactosidase activity (U) was determined by measuring absorption at 420 nm, using a reference solution without bacteria.

Table 17: The composition of initial incubation mixture (Plate A) in the classic SOS/*umuC* test.

(Plate A)	Without metabolic activation (- S9)	With metabolic activation (+ S9)
Blind control	10 μL bidistilled water 90 μL 1x TGA medium	10 μL bidistilled water 90 μL 1x TGA medium
Solvent control	10 μL 10% DMSO 90 μL bacterial suspension	10 μL 10% DMSO 90 μL 10% S9 mix
Negative control	10 μL bidistilled water 90 μL bacterial suspension	10 μL bidistilled water 90 μL 10% S9 mix
Positive control	10 μL NQNO (5 μg mL^{-1}) 90 μL bacterial suspension	10 μL AFB1 (5 μg mL^{-1}) 90 μL 10% S9 mix
Sample	10 μL benzophenone 90 μL bacterial suspension	10 μL benzophenone 90 μL 10% S9 mix

3.3.4.2 Modified SOS/*umuC* test

In the modified SOS/*umuC* test, the bacterial culture was incubated overnight with shaking at 37 °C, then diluted 10-times with fresh 1x TGA medium and again incubated at 37 °C for 1.5 h with shaking until the bacteria reached the exponential growth phase.

Aqueous solutions of each benzophenone (100 mg L^{-1} and 1 μg L^{-1}) were exposed to MP/UV light, where samples were taken after 0, 7 and 24 h of irradiation. Aqueous samples of benzophenone photodegradation mixtures were then transferred to a microtiter plate.

The initial incubation mixture (Plate A) consisted of 180 μL of the water sample, 20 μL of 10x TGA and 70 μL of bacterial culture. In the case of metabolic activation, 180 μL of water sample was incubated along with 20 μL of 10x TGA with cofactors and 70 μL of the S9 bacterial culture mixture, prepared as described in the ISO standards (450 μL of S9 was added to 15 mL of the bacterial culture). In addition, blind, solvent, negative and positive controls were used (Table 18).

The microtiter plate was incubated at 37 °C for 2 h with shaking at 150 rpm. The incubation mixture was then diluted 10-times with fresh warm 1x TGA medium (Plate B), following a subsequent additional incubation period of 2 h at 37 °C. The bacterial growth rate (G) was determined by measuring absorbance at 600 nm.

30 µL of the sample was then transferred to another microtiter plate (Plate C) containing 120 µL of B-buffer, and immediately, 30 µL of ONPG was added. After 30 min of shaking at 25 °C using ONPG as a substrate, 120 µL of stop reagent (Na_2CO_3) was added. ß-galactosidase activity (U) was determined by measuring absorption at 420 nm, using a reference solution without bacteria.

Table 18: The composition of initial incubation mixture (Plate A) in the modified SOS/*umuC* test.

(Plate A)	Without metabolic activation (- S9)	With metabolic activation (+ S9)
Blind control	180 µL bidistilled water 20 µL 10x TGA medium 70 µL 1x TGA medium	180 µL bidistilled water 20 µL 10x TGA medium with cofactors 70 µL 1x TGA medium with S9 mix
Solvent control	27 µL 30% DMSO 153 µL sterile water 20 µL 10x TGA medium 70 µL bacterial suspension	27 µL 30% DMSO 153 µL sterile water 20 µL 10x TGA medium with cofactors 70 µL bacterial suspension with S9 mix
Negative control	180 µL bidistilled water 20 µL 10x TGA medium 70 µL bacterial suspension	180 µL bidistilled water 20 µL 10x TGA medium with cofactors 70 µL bacterial suspension with S9 mix
Positive control	180 µL NQNO (0.25 µg mL^{-1}) 20 µL 10x TGA medium 70 µL bacterial suspension	180 µL AFB1 (5 µg mL^{-1}) 20 µL 10x TGA medium with cofactors 70 µL bacterial suspension with S9 mix
Sample	180 µL aqueous sample 20 µL 10x TGA medium 70 µL bacterial suspension	180 µL aqueous sample 20 µL 10x TGA medium with cofactors 70 µL bacterial suspension with S9 mix

3.3.4.3 Calculation of the results

The bacterial growth rate (G) was calculated using the following formula:

$$G = \frac{OD_{600}\ (\text{sample}) - OD_{600}\ (\text{blank})}{OD_{600}\ (\text{control}) - OD_{600}\ (\text{blank})} \quad (1)$$

A growth ratio of < 0.5, representing > 50% inhibition of the biomass growth, was considered to be indicative of samples being cytotoxic.

ß-galactosidase activity (U) in relative units was calculated from the formula:

$$U = \frac{OD_{420}\ (\text{sample}) - OD_{420}\ (\text{blank})}{OD_{600}\ (\text{sample}) - OD_{600}\ (\text{blank})} \quad (2)$$

Induction ratio (IR) was calculated by the equation:

$$IR = \frac{1}{G} \times \frac{OD_{420}\ (\text{sample}) - OD_{420}\ (\text{blank})}{OD_{420}\ (\text{control}) - OD_{420}\ (\text{blank})} \quad (3)$$

An induction ratio of ≥ 2 (for pure compounds) and ≥ 1.5 (for un-concentrated water samples) (ISO, 2000) was taken as the threshold at which the sample was considered as genotoxic.

3.3.4.4 Schematic presentation of SOS/*umuC* test

Figure 13 presents the procedure for testing the genotoxic potential of benzophenones and their photodegradation mixtures by using the SOS/*umuC* assay.

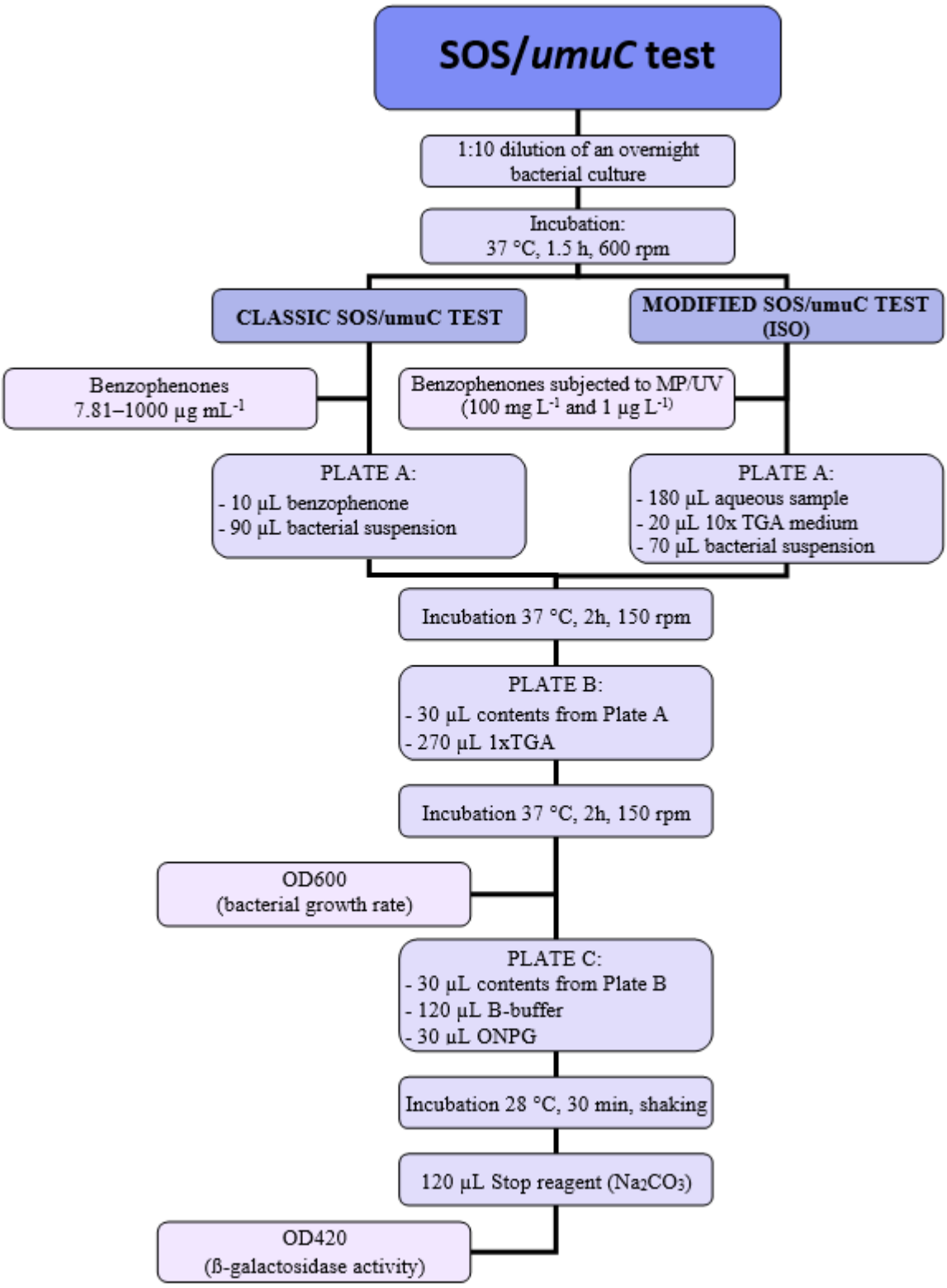


Figure 13: Schematic presentation of the classic and modified SOS/*umuC* assay for testing genotoxicity of the parent benzophenones and their photodegradation mixtures.

4 Results and Discussion

4.1 Development of Analytical Method for Detection of Benzophenones in the Environmental Samples

To develop an analytical method for detecting selected benzophenone derivatives in aqueous and sediment samples, the following steps were considered as crucial to optimize: solid phase extraction of water samples, microwave extraction of sediment samples, derivatization, and instrumental analysis using GC-MS. To achieve good sensitivity and accurate results, these steps have been thoroughly investigated, the most efficient conditions for analysis of the target benzophenones determined and the performance of the method validated.

4.1.1 Solid phase extraction of aqueous samples

Extraction of aqueous samples was performed by SPE using Oasis HLB sorbent, which enables retention of the investigated benzophenones by exhibiting both hydrophilic and lipophilic characteristics. To optimize the SPE procedure, specific physical and chemical characteristics of the analyte, sorbent and solvents must be considered. To achieve the most efficient extraction of benzophenones from different aqueous matrices, the effects of sample volume and pH, elution solvent, and matrix effects were evaluated. The tested parameters are presented in Figure 14.

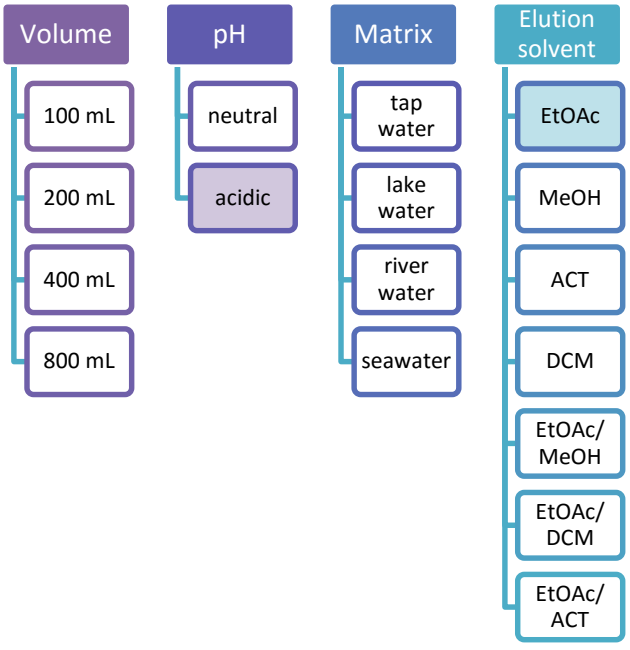


Figure 14: Tested parameters in SPE optimization.

The effect of sample volume on extraction efficiency was determined by using different amounts of tap water (100, 200, 400 and 800 mL). No breakthrough occurred for any of the

analytes when larger volumes were used and SPE efficiencies were all ≥ 80% (Table 19). This confirms the method's suitability for extracting large volumes. Hence, in order to detect low concentrations of benzophenones in environmental samples, a volume of 800 mL was used.

Table 19: The effect of sample volume on SPE efficiency (samples spiked at 200 ng L^{-1}; n = 3).

Analyte	SPE efficiency (%)			
	V = 100 mL	V = 200 mL	V = 400 mL	V = 800 mL
BP	80 ± 2	83 ± 6	88 ± 4	86 ± 5
H-BP	93 ± 4	95 ± 3	90 ± 4	88 ± 6
DH-BP	89 ± 2	81 ± 6	86 ± 1	85 ± 3
HM-BP	95 ± 1	90 ± 1	101 ± 3	97 ± 4
DHM-BP	85 ± 4	80 ± 4	86 ± 1	80 ± 7
KP	92 ± 5	97 ± 7	101 ± 6	84 ± 1
EtBP	88 ± 3	85 ± 4	98 ± 9	82 ± 5
AcBP	90 ± 8	88 ± 9	87 ± 9	81 ± 9

The influence of pH on the SPE efficiency was investigated by comparing extraction efficiency of SPE performed under neutral and acidic conditions. Basic conditions have not been tested since acidic pharmaceuticals such as ketoprofen (pK_a = 4.45) are known to dissociate in a basic (and neutral) environment, leading to reduced adsorption on the SPE sorbent, while other benzophenones have their pK_a values between 6.99–7.56 (Table 2), thus the matrix pH was not expected to significantly affect their retention. In addition, studies report that an acidic pH enables the optimal SPE performance for KP and HM-BP (Cuderman & Heath, 2007; Giokas et al., 2004; Kosjek et al., 2005). Table 20 shows how acidic conditions resulted in equal or higher extraction yields of the investigated compounds, most notably for ketoprofen. Therefore, the benzophenone samples were acidified to pH 2–3 prior to SPE.

Table 20: Comparison of benzophenones SPE extraction efficiency in acidic and neutral conditions (samples spiked at 200 ng L^{-1}; n = 3).

Analyte	SPE efficiency (%)	
	pH = 2–3	pH = 7–8
BP	88 ± 4	84 ± 7
H-BP	90 ± 4	81 ± 5
DH-BP	86 ± 1	78 ± 3
HM-BP	101 ± 3	93 ± 9
DHM-BP	86 ± 1	80 ± 8
KP	101 ± 6	78 ± 3
EtBP	98 ± 9	97 ± 4
AcBP	87 ± 9	87 ± 7

To determine the optimal solvent for elution of benzophenones, the following solvents or solvent mixtures were tested: EtOAc, MeOH, ACT, DCM, EtOAc/MeOH (1:1, v/v),

EtOAc/DCM (1:1, v/v) and EtOAc/ACT (1:1, v/v), which are presented in Figure 15. Although MeOH and the solvent mixtures showed good results for individual compounds, EtOAc provided optimal extraction efficiencies for simultaneous elution of all the target compounds. To determine the optimal amount of the elution solvent that would efficiently remove analytes from the sorbent using the smallest volume capable of yielding reproducible extraction efficiencies, two combinations were tested: 4 aliquots of 450 µL EtOAc and 4 aliquots of 1 mL EtOAc. Since there was no trace of analytes in the eluant after 1.8 mL of elution solvent, EtOAc applied in 4 aliquots of 450 µL was considered optimal.

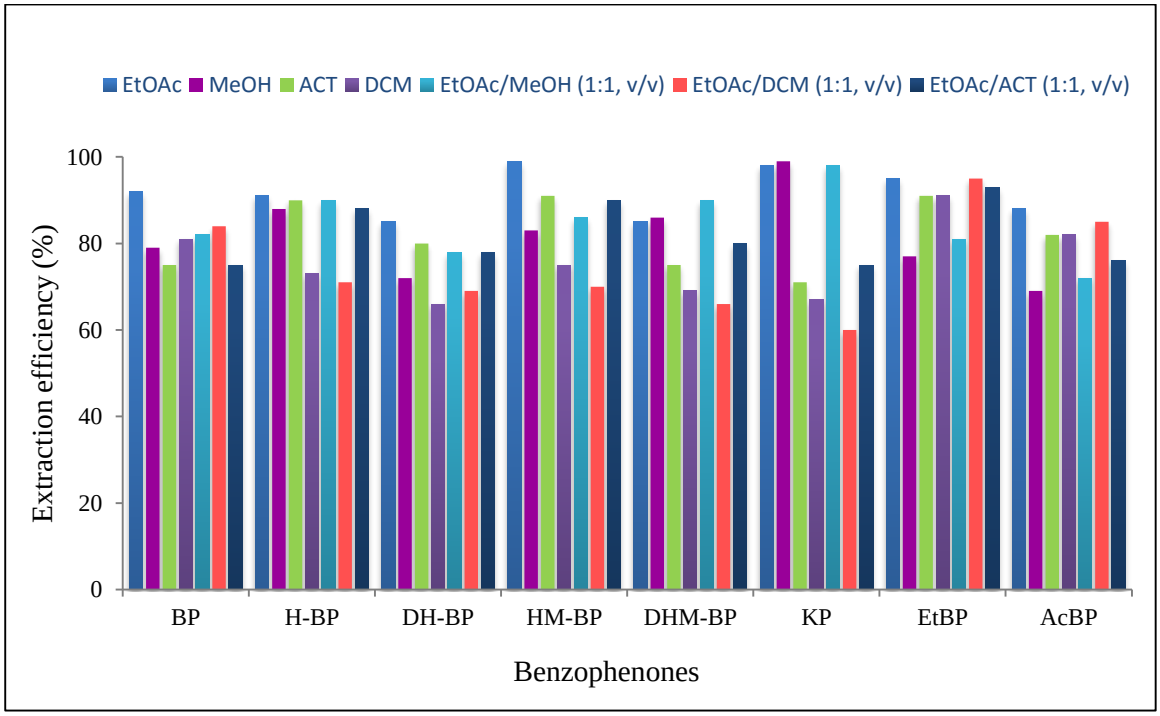


Figure 15: Influence of elution solvent on extraction efficiency of target benzophenones from tap water at spike level of 500 ng L^{-1}.

The effect of different aqueous matrices on SPE efficiency was investigated by comparing results for spiked tap water, river, lake and seawater (the blank samples were collected at sites considered pristine and analysis revealed all of the target compounds were below the LOD). Although the presence of KCl in aqueous samples was reported to increase the extraction yield of HM-BP (Giokas et al., 2004), no significant change in extraction efficiency of the target compounds in seawater was observed. Results revealed SPE efficiencies above 85% for all the investigated surface waters (Table 21) with < 10% RSD. Furthermore, the use of internal standards, added to the sample prior to SPE, took into account any significant influence on the method performance.

Table 21: SPE efficiencies of the target analytes with their standard deviations (n = 3) for different aqueous matrices spiked at 200 ng L^{-1} (V = 400 mL).

Analyte	SPE efficiency (%)			
	Tap water	River	Lake	Sea
BP	88 ± 4	84 ± 4	91 ± 2	88 ± 1
H-BP	90 ± 4	92 ± 3	92 ± 3	99 ± 1
DH-BP	86 ± 1	85 ± 3	89 ± 4	93 ± 3
HM-BP	101 ± 3	101 ± 1	98 ± 2	108 ± 1
DHM-BP	86 ± 1	89 ± 6	93 ± 7	95 ± 3
KP	101 ± 6	94 ± 4	99 ± 2	90 ± 3
EtBP	98 ± 9	91 ± 9	101 ± 3	101 ± 1
AcBP	87 ± 9	85 ± 8	88 ± 9	91 ± 7

4.1.2 Microwave assisted extraction of sediment samples

The method development for extraction of benzophenones from sediment involved determining extraction efficiencies of spiked sediment samples, subjected to two different procedures: ultrasound and microwave assisted extraction. Since MAE offers many advantages over other techniques, the main goal was to determine the optimal conditions for this type of extraction. For this purpose, the effect of extraction solvent, temperature, irradiation time and microwave power on extraction yield was investigated. The evaluated parameters are presented in Figure 16.



Figure 16: Parameters for MAE optimization.

Different solvents were tested based on their dielectric constant, polarity and data from previously published studies (Antonić & Heath, 2007; Kameda et al., 2011; Sánchez-Brunete et al., 2011). Among the solvents tested (MeOH, EtOAc and ACT), EtOAc was the least efficient at extracting benzophenones (Figure 17), which may be attributed to its low dielectric constant (ε = 6.0) compared to MeOH (ε = 33.6) and ACT (ε = 20.7) (McMurry, 1988). Since MeOH gave the best results, it was further investigated as a component of different solvent mixtures: MeOH/EtOAc (1:1, v/v), MeOH/DCM (1:1, v/v) and MeOH/ACT (1:1, v/v). The latter mixture provided the highest extraction efficiencies for most of the target compounds (Figure 17). Further, the addition of an acid (pH = 1–2) significantly enhanced extraction efficiency (Figure 17). A similar observation was reported for KP by Antonić & Heath (2007). When comparing the effect of acetic and formic acid, no significant difference was observed, thus a mixture of MeOH and ACT (1:1, v/v) with the addition of 5% (w/w) formic acid was used.

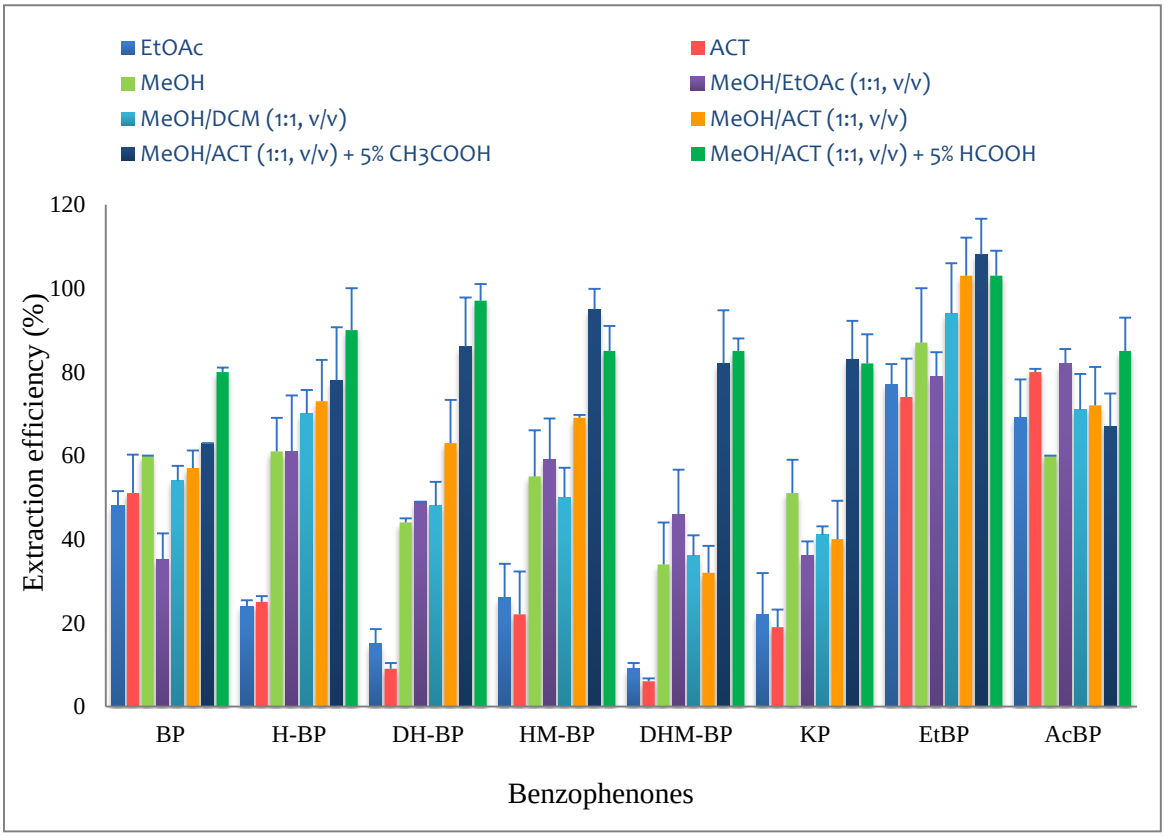


Figure 17: Effect of solvent on MAE of target benzophenones from sediments at 50 ng g^{-1} (n = 2).

To investigate the effect of temperature on MAE efficiency, temperatures of 25–175 °C were tested (Figure 18). Increasing the temperature from 25 °C to 150 °C enhanced extraction by up to 29%. Further heating to 175 °C led to a slight decrease in extraction yield for some of the benzophenones (Figure 18A), which may be attributed to the degradation of the compounds at higher temperatures. For these reasons, the optimal temperature for extraction was set at 150 °C. Increasing irradiation time from 10 min to 30 min resulted in slightly higher extraction efficiencies, but extended extraction times (up to 60 min) had no effect (Figure 18B). Therefore, an extraction time of thirty minutes was chosen as optimal. Increasing microwave power from 400 to 1600 W did not significantly enhance extraction efficiency (Figure 18C). However, in a closed vessel system, power settings strongly depend on the number of samples being simultaneously extracted (Camel,

2000). For this reason, and to avoid excessive heating and overpressure, 800 W of power was selected.

In summary, optimal conditions for extraction of the investigated benzophenones from sediment involved MAE for 30 min at 150 °C with a power of 800 W. By using these conditions, extraction efficiencies of 71% to 106% were obtained at the four fortification levels (Table 22).

Table 22: MAE efficiencies of the target benzophenones from sediment samples at different fortification levels (n = 3).

Analyte	**MAE efficiency (%)**			
	25 ng g^{-1}	**50 ng g^{-1}**	**100 ng g^{-1}**	**500 ng g^{-1}**
BP	106 ± 4	103 ± 1	102 ± 1	87 ± 2
H-BP	99 ± 2	90 ± 4	91 ± 2	83 ± 3
DH-BP	100 ± 5	97 ± 4	97 ± 2	81 ± 1
HM-BP	74 ± 4	85 ± 6	75 ± 1	85 ± 4
DHM-BP	83 ± 1	85 ± 3	71 ± 1	80 ± 3
KP	80 ± 2	82 ± 7	78 ± 2	79 ± 4
EtBP	85 ± 9	85 ± 6	99 ± 1	78 ± 6
AcBP	91 ± 10	80 ± 8	91 ± 7	99 ± 2

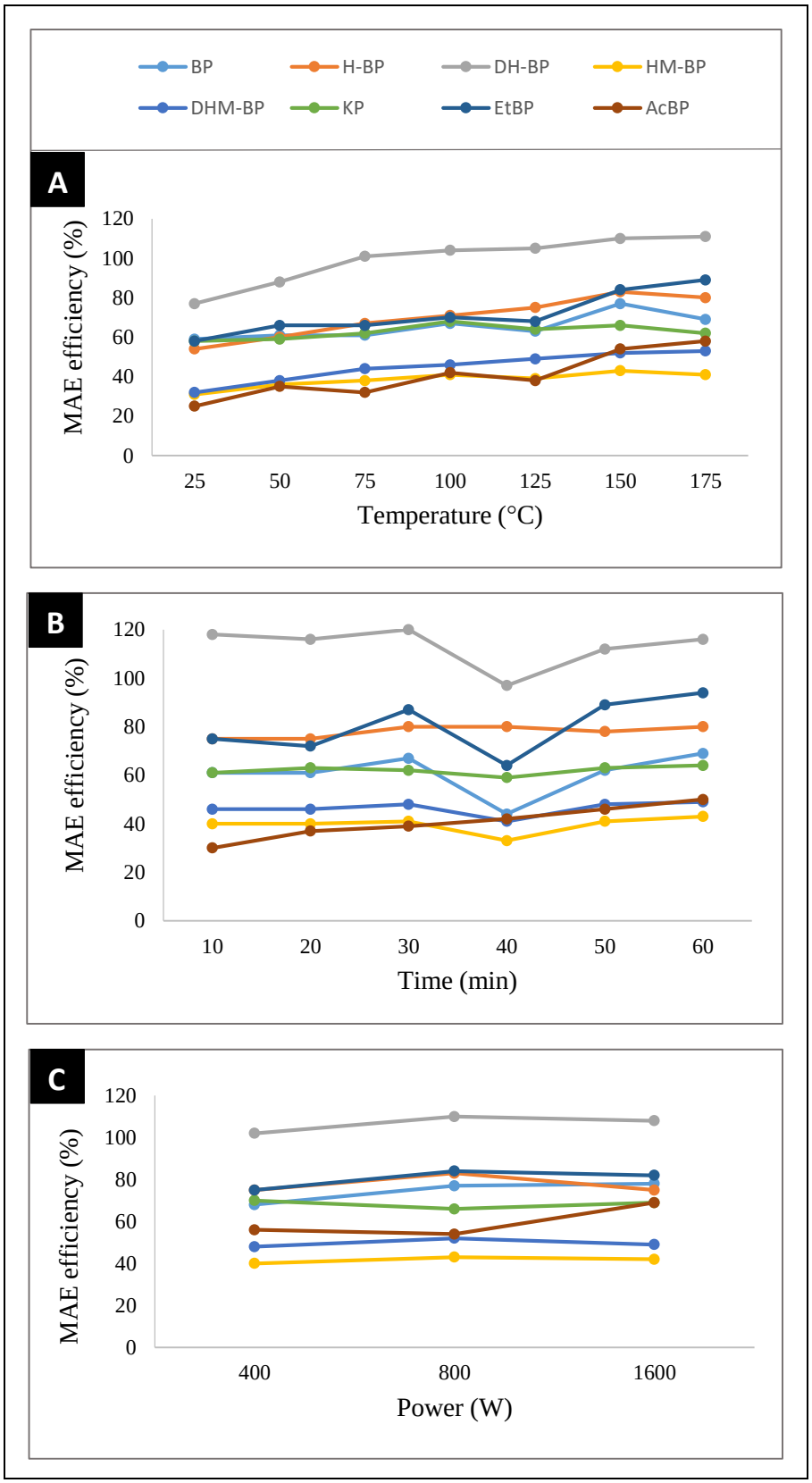


Figure 18: Influence of irradiation temperature (A), time (B) and power (C) on extraction efficiency of benzophenones from sediments by MAE at 500 ng g^{-1}.

Since most published studies (Kameda et al., 2011; Löffler & Ternes, 2003; Sánchez-Brunete et al., 2011) involve the use of ultrasound for extracting benzophenone derivatives, a comparison was made between the efficiency of MAE and UAE. Using an ultrasonic water bath (VWR, Model 550D, VWR International, West Chester, PA, USA) different temperatures (25, 40 and 60 °C) and reaction times (10, 30 and 50 min) were tested to determine the optimal conditions, which were set at 40 °C for 30 min. Using the same solvent mixture as for MAE, sediment samples (4 g) were sonicated for 30 min at 40 °C. MAE gave higher extraction efficiencies for all the investigated compounds (Figure 19). This agrees with Antonić and Heath (2007), who report significant improvement in the extraction efficiency of ketoprofen from sediment by microwave extraction compared to ultrasonic extraction (Antonić & Heath, 2007).

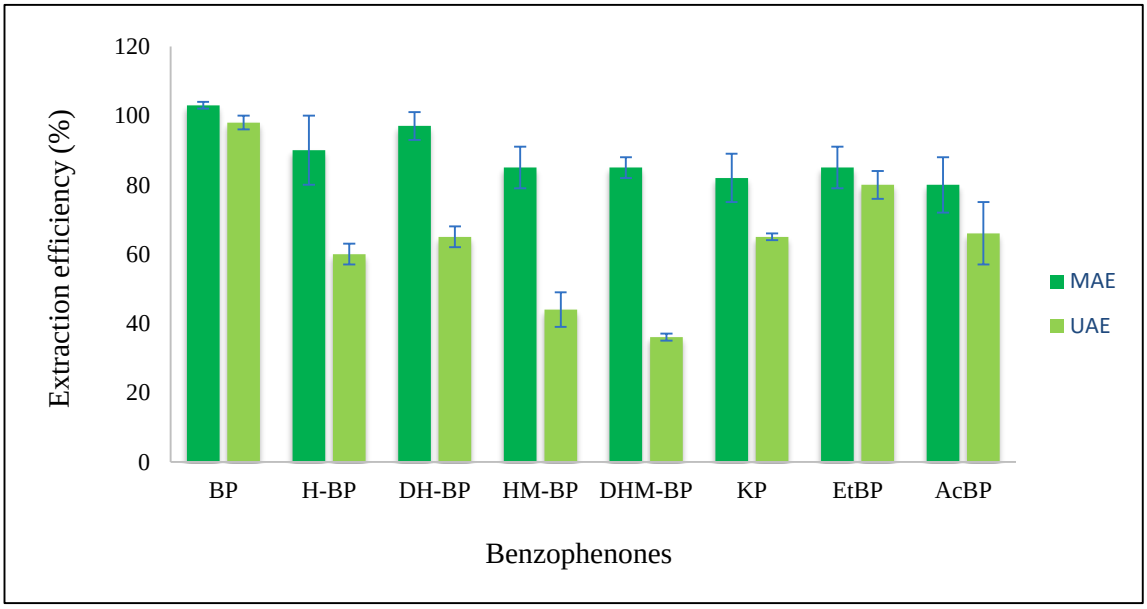


Figure 19: Comparison of extraction efficiencies for MAE and UAE of the target compounds at a spike level of 50 ng g^{-1} (n = 3).

Finally, a clean-up step was included in the procedure in order to minimize interferences. Different methods for clean-up have been reported for benzophenones, including the use of silica (Kameda et al., 2011; Rodil & Moeder, 2008b) and other SPE sorbents, such as reversed-phase (Antonić & Heath, 2007) and mixed-mode sorbents (Löffler & Ternes, 2003). Since the optimized SPE method for extracting benzophenones from water samples resulted in efficient extraction (Table 21), the same Oasis HLB reversed phase sorbent was used to clean-up sediment extracts.

4.1.3 Derivatization

Derivatization procedure was evaluated in terms of choosing the optimal derivatization agent and reaction conditions. The presence of a carbonyl group in the chemical structure of benzophenones allows the use of PFBHA as a derivatization reagent, which converts the carbonyl group to an oxime. Using PFBHA, optimization involved testing several solvents (MeOH, ACN, HEX), reaction temperature (40–100 °C), reaction time (30 min–24 h) and the volume of added PFBHA solution (150–600 µL) (Figure 23).

Since derivatized standards of the selected compounds were commercially unavailable, it was not possible to calculate the derivatization efficiency. However, aliquots of the sample were analyzed at selected time intervals until no further increase in product peak(s)

was observed (Figure 20, Figure 22) and derivatization was assumed stable. In addition, for semi-volatile compounds (BP, EtBP and AcBP), the peaks of underivatized compounds were monitored, i.e. the compound was considered completely derivatized when the underivatized peak was undetectable. PFBHA proved to be an efficient derivatization reagent for BP, EtBP, AcBP and the internal standards BP-d_5 and iPrBP, while for the other benzophenones silylation resulted in an improved chromatographic response. Since it was not possible to achieve simultaneous derivatization of all investigated compounds, sample extracts were halved and derivatized separately.

Choosing the optimal solvent for derivatization of BP, EtBP and AcBP involved testing MeOH, ACN and HEX. Since MeOH gave the best results (Figure 21A), PFBHA was dissolved in MeOH and the PFBHA solution was used in further analysis for derivatization. Investigation of reaction time revealed that BP and EtBP require 15 h to achieve maximum derivatization (Figure 22A), while AcBP needed 1 h, but began to decompose with further incubation (Figure 22B). A temperature of 60 °C was the most efficient, since further heating affected the stability of the compounds, AcBP in particular (Figure 20A). In summary, the optimal conditions for derivatization of BP, EtBP and AcBP included the addition of 300 µL of PFBHA in methanol at 60 °C.

For derivatizing H-BP, DH-BP, HM-BP, DHM-BP, KP and internal standards HM-BP-d_5 and KP-d_3, which contain the OH group, three silylation reagents (MSTFA, MTBSTFA and BSTFA) were investigated. Both MSTFA and BSTFA efficiently derivatized these compounds, producing responses of similar intensity, while MTBSTFA showed significantly lower chromatographic responses for all benzophenones with the exception of KP. MTBSTFA derivatives have greater thermal and hydrolytic stability compared to other trimethylsilyl derivatives (Kosjek et al., 2011), but the low reactivity of MTBSTFA (Farré et al., 2007) may be the reason for the incomplete derivatization of the phenolic groups. Since the reaction of MSTFA with KP gave a satisfactory derivatization yield, MSTFA was selected for derivatizing hydroxylated benzophenones.

Using MSTFA, different experimental conditions were tested (Figure 23) including reaction temperature (40–80 °C), time (30 min–3 h), type of solvent (EtOAc, ACN, ACT, HEX), amount of solvent (300–600 µL) and amount of derivatization agent (30 µL and 60 µL). EtOAc gave the best results (Figure 21B). Increasing the reaction temperature to 60 °C resulted in improved abundance, while further elevation of temperature did not have any significant effect on derivatization yield nor on the stability of benzophenones (Figure 20B). In addition, testing the reaction time revealed that the compounds are effectively derivatized in 60 minutes (Figure 22C). To conclude, the optimal conditions for derivatization of benzophenones with hydroxyl moieties were as follows: the dry extract was dissolved in 300 µL of EtOAc, 30 µL of MSTFA were added and left to derivatize for 1 h at 60 °C.

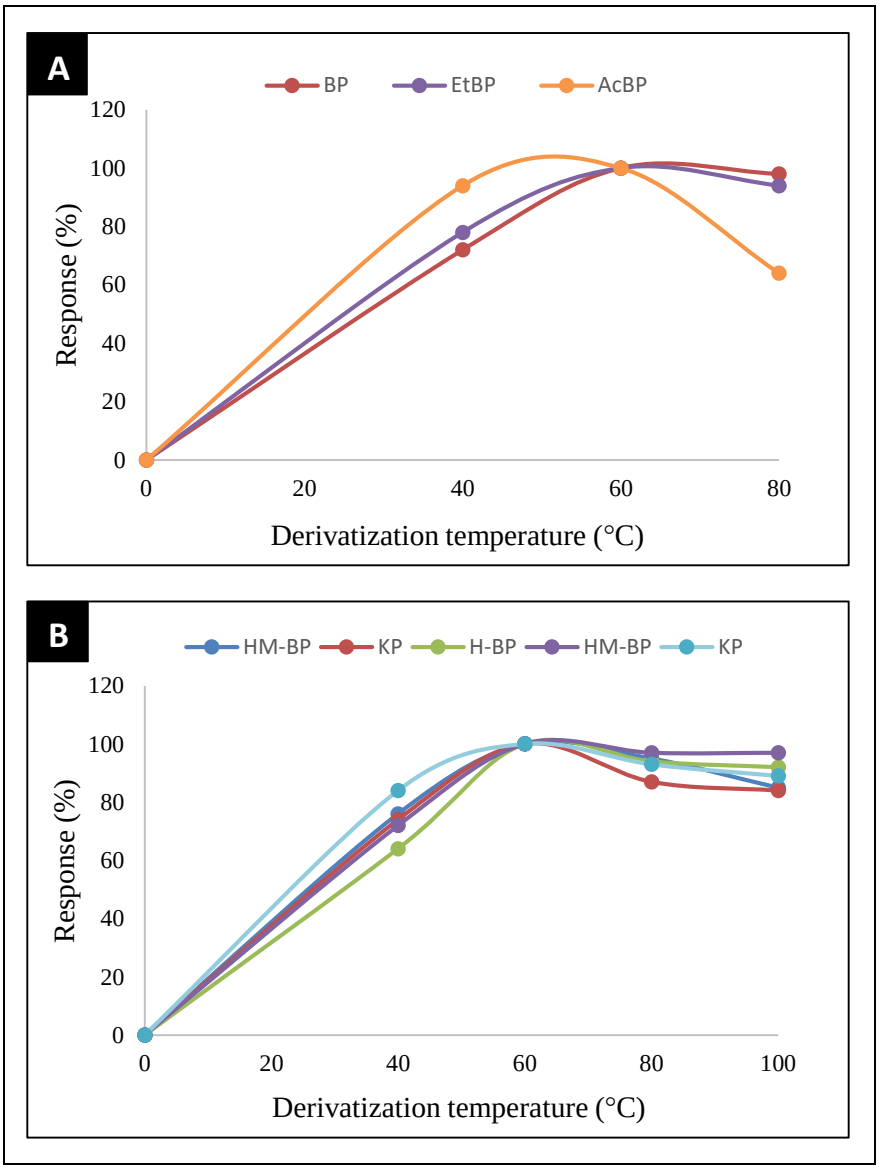


Figure 20: Influence of reaction temperature on derivatization yield of BP, EtBP and AcBP using PFBHA (A) and hydroxylated benzophenone derivatives using MSTFA (B).

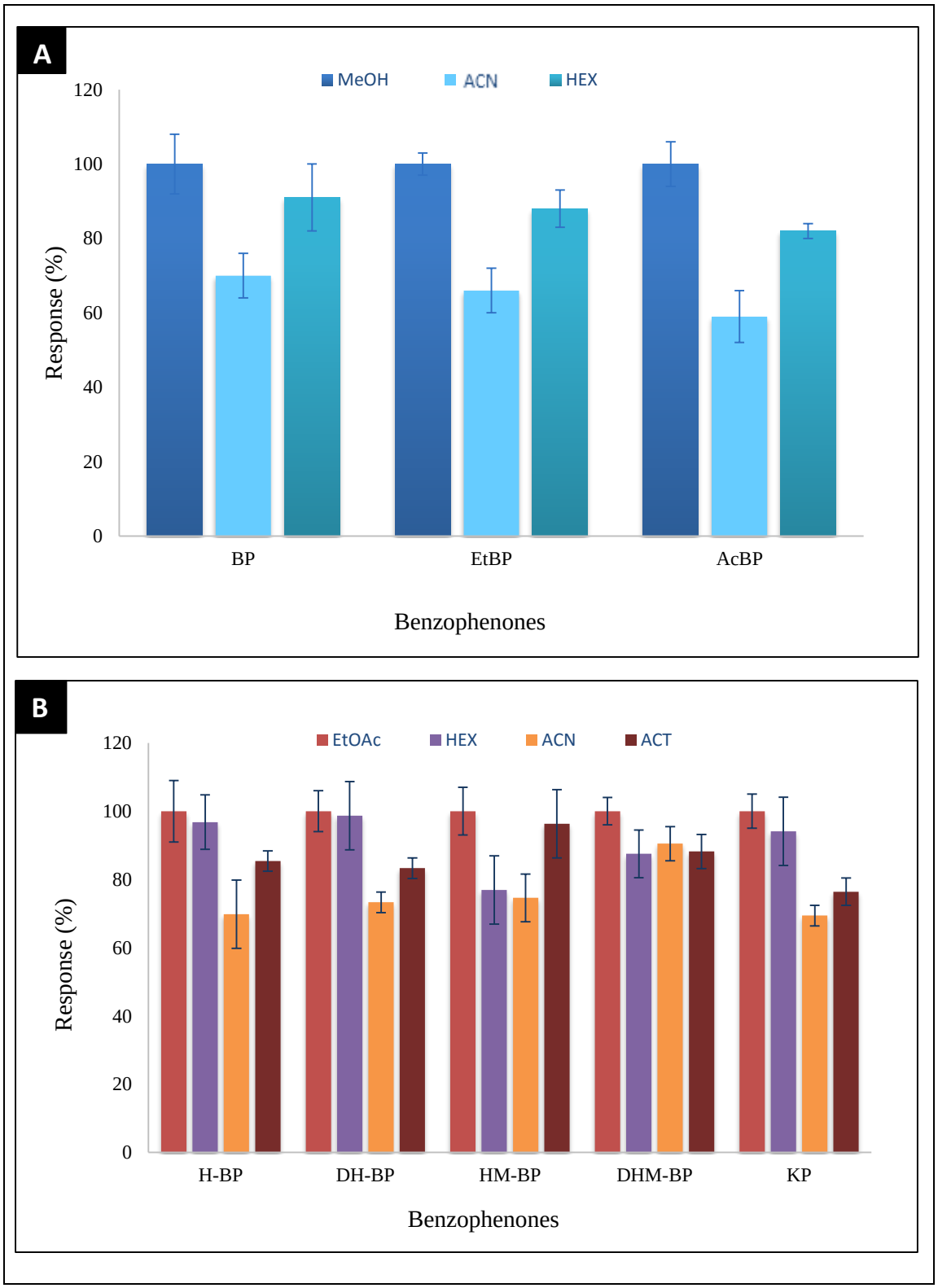


Figure 21: Effect of solvent (MeOH, ACT and HEX) on the derivatization of three benzophenones (BP, EtBP and AcBP) using PFBHA (responses are normalized to MeOH; n = 3) (A) and the influence of solvent (EtOAc, HEX, ACN and ACT) on the derivatization of five hydroxylated benzophenones using MSTFA (responses are normalized to EtOAc; n = 3) (B).

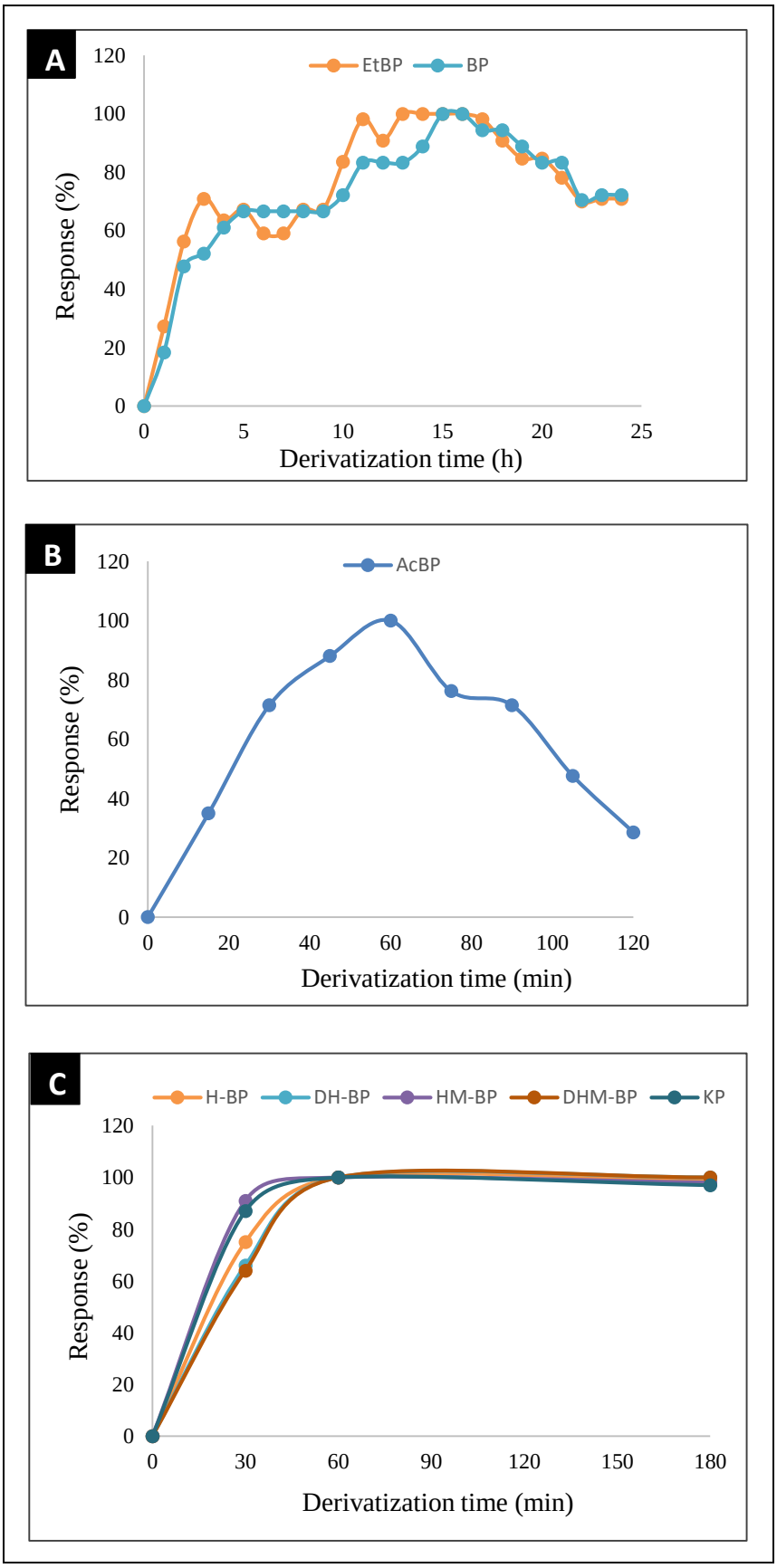


Figure 22: Influence of reaction time on derivatization yield of BP and EtBP (A) and AcBP (B) at 60°C in MeOH using PFBHA and benzophenones including hydroxyl moieties (C) at 60°C in EtOAc using MSTFA.

Analyte	Derivat. agent	Amount of derivat. agent (µL)	Solvent	Amount of solvent (µL)	Reaction temp. (°C)	Reaction time (h)
BP, EtBP, AcBP	PFBHA	150 **300** 600	**MeOH** ACN HEX	/	40 **60** 80 100	0.5 **1** 3 **15** 24
KP, H-BP, DH-BP, HM-BP, DHM-BP,	MSTFA	**30** 60	**EtOAc** ACN ACT HEX	**300** 600	40 **60** 80	0.5 **1** 3

Figure 23: Tested parameters for determining optimal derivatization conditions of benzophenones (optimal conditions are in bold).

4.1.4 GC-MS analysis

The retention times, quantification and identification ions of the target compounds are listed in Table 23. Since derivatization of KP, H-BP, HM-BP, DH-BP and DHM-BP using MSTFA has already been reported (Jeon et al., 2006; Kosjek et al., 2011; Negreira et al., 2009a; Sebők, Vasanits-Zsigrai, Palkó, Záray, & Molnár-Perl, 2008), this study only confirmed their silylation products in full-scan mode. For BP, EtBP and iPrBP, molecular ions 377, 405 and 419 were the most abundant ions, respectively. The most abundant ion for AcBP was *m/z* 419.

Derivatization of EtBP and iPrBP with PFBHA resulted in the formation of *cis/trans* isomers at the oxime bond, yielding double peaks in the chromatogram. The formation of *cis/trans* isomers has been previously reported by derivatization of asymmetric carbonyl compounds using PFBHA (Nawrocki et al., 1996; Strassnig, Wenzl, & Lankmayr, 2000). The ratio of the isomers by derivatization of EtBP and iPrBP has been investigated and was determined as stable under the given analytical conditions (1.02 ± 0.02 for EtBP and 0.91 ± 0.03 for iPrBP). Hence, only one isomer peak was used for quantification (Table 23).

The studied compounds were detected and quantified by monitoring their most abundant ions (Table 23). To confirm their identity, the ion ratios for a given analyte measured as the peak area of a quantification ion divided by the peak area of the identification ion, was required to be within ± 20% of the reference compounds' ion ratios. Retention times of the analytes had to be within ± 0.20 min of the reference compounds' average retention time.

Table 23: Derivatization agents, retention times (t_R), quantification and identification ions (*m/z*) used for quantitative analysis of the target compounds.

Analyte	Derivatization reagent	t_R (min)	Quantification ion (*m/z*)	Identification ions (*m/z*)
BP	PFBHA	9.99	377	360, 196
H-BP	MSTFA	8.37	270	255, 193
DH-BP	MSTFA	9.17	343	358, 271
HM-BP	MSTFA	8.71	285	300, 242
DHM-BP	MSTFA	9.97	373	388, 299
KP	MSTFA	9.69	282	311, 295
EtBP	PFBHA	10.8	405	388, 224
AcBP	PFBHA	12.9	419	161, 105
iPrBP	PFBHA	11.0	419	376
BP-d_5	PFBHA	9.98	382	365
HM-BP-d_5	MSTFA	8.69	290	305
KP-d_3	MSTFA	9.67	285	314

4.1.5 Performance of the analytical method

Validation of the analytical method for surface waters was performed using tap water; however, limits of detection and SPE efficiencies were demonstrated by using blank samples from different surface water matrices (river, lake and seawater) and sediments, collected from locations considered pristine. The presence of the target analytes in blank samples was < LOD.

The results of method validation are presented in Table 24. The good linearity of the method is expressed with r^2 values ≥ 0.998 in both matrices. The exception is AcBP (r^2 = 0.980) in an aqueous matrix. Linearity was determined between 2–1500 ng L^{-1} (KP: 2–3000 ng L^{-1}) for water samples, and 2–500 ng g^{-1} (BP: 2–1000 ng g^{-1}) for sediment samples. LOD were between 0.19 ng L^{-1} and 1.9 ng L^{-1} in water samples (lake, river and seawater) and between 0.1 ng g^{-1} and 1.4 ng g^{-1} in sediments. The method also gave good accuracy (95–106%) for both matrices, which was determined at spiked concentrations of 500 ng L^{-1} and 1500 ng L^{-1} for water samples and at 50 ng g^{-1} and 500 ng g^{-1} for sediment samples. SPE efficiency was performed at two concentration levels (200 ng L^{-1} and 1000 ng L^{-1}) of spiked tap water. Table 24 shows their average efficiencies, which are ≥ 91% for each analyte. By comparing the SPE efficiencies of tap, river, lake and seawater, it is apparent that the efficiency is unaffected by the nature of the matrix (see Section 4.1.1). The efficiency of MAE was performed at 25, 50, 100 and 500 ng g^{-1}. The values given in Table 24 represent average values, which all are ≥ 80%. The instrumental repeatability was expressed as the RSD of triplicate injections of each sample at 200 ng L^{-1} and 1000 ng L^{-1}, and was 1.7–4.3% and 2.2–5.8%, respectively. Analytical methods for both water and sediment samples showed good repeatability, which was determined at 500 ng L^{-1} and 1500 ng L^{-1} for water samples and at 50 ng g^{-1} and 500 ng g^{-1} for sediment samples (Table 24).

Table 24: Validation of analytical method for the determination of the target benzophenone derivatives in water and sediment samples.

Analyte	Efficiency (%)		Method Repeatability (%)		Linearity (r^2)		Accuracy (%)		Limits of detection (LOD) and quantification (LOQ)									
									Tap water (ng L^{-1})		Surface water (ng L^{-1})						Sediment (ng g^{-1})	
											River		Lake		Sea			
	SPE	MAE	Water	Sediment	Water	Sediment	Water	Sediment	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
BP	91 ± 4	99 ± 8	3.5–5.3	0.5–2.0	0.999	0.999	101	106	1.3	4.3	1.9	6.3	1.6	5.3	1.6	5.3	1.4	4.5
H-BP	95 ± 7	91 ± 7	3.7–4.8	4.1–11.5	0.998	0.999	105	103	0.4	1.4	0.3	1.1	0.1	0.5	0.3	0.5	0.5	1.7
DH-BP	93 ± 9	94 ± 9	5.0–5.9	0.7–3.6	0.999	0.999	102	103	0.9	3.2	0.6	1.8	0.4	1.4	0.3	1.4	0.3	0.9
HM-BP	95 ± 9	80 ± 6	0.8–2.0	4.1–7.0	0.999	0.999	103	101	0.7	2.3	0.8	2.7	0.6	2.0	0.5	2.0	0.1	0.2
DHM-BP	91 ± 6	80 ± 6	3.0–5.2	3.2–3.3	0.999	0.999	99.4	104	0.4	1.4	0.2	0.8	0.1	0.4	0.2	0.4	0.3	0.9
KP	96 ± 7	80 ± 2	1.8–2.3	5.3–8.1	0.998	0.999	100	98.7	0.8	2.8	0.4	1.4	0.5	1.8	0.5	1.8	1.4	4.8
EtBP	93 ± 8	87 ± 9	3.0–5.5	7.0–7.2	0.998	0.999	98.4	103	0.7	2.4	1.5	5.1	1.1	3.7	1.7	3.7	1.0	3.3
AcBP	94 ± 9	90 ± 8	5.9–9.6	1.8–9.9	0.980	0.998	110	95.3	1.3	4.5	1.4	4.7	0.7	2.3	1.3	2.3	1.2	3.8

4.2 Occurrence of Benzophenones in Environmental Samples

The developed method was applied to the analysis of 14 water and sediment samples collected from different locations in Slovenia (13) and Croatia (1), representing either areas with a high intensity of water related recreational activities (5 lakes, 3 rivers and 3 coastal sea sites) or receiving water bodies of WWTPs (3 rivers). Sample locations are presented in Figure 8.

Table 25 (surface waters) and Table 26 (sediment samples) show the determined concentrations of the target benzophenones in the samples. It must be emphasized that sampling was performed during summer, which implies the highest use of sunscreen products; moreover, urban wastewaters were not diluted by storm waters and rivers had their lowest flow rates. These samples are therefore representative of a worst-case scenario.

Table 25: Concentrations (ng L^{-1}) of eight benzophenone derivatives in surface water samples.

Matrix	Sampling site	BP	H-BP	DH-BP	HM-BP	DHM-BP	KP	EtBP	AcBP
River	Kolpa	61	<LOD	11	120	<LOD	<LOD	<LOD	<LOD
	Krka	30	<LOD	1.9	9.4	<LOD	<LOD	<LOD	<LOD
	Nadiža	88	<LOD	<LOD	30	<LOD	<LOD	<LOD	<LOD
Lake	Bled	120	8.4	25	690	6.5	<LOD	<LOD	<LOD
	Šobec	120	7.2	130	820	4.5	<LOD	<LOD	<LOD
	Rakitna	33	<LOD	29	110	2.6	<LOD	<LOD	<LOD
	Bohinj	35	<LOD	<LOD	43	<LOD	<LOD	<LOD	<LOD
	Bloke	48	<LOD	2.9	38	<LOD	<LOD	<LOD	<LOD
Sea	Portorož	52	4.1	8.7	380	5.3	<LOD	<LOD	<LOD
	Ankaran	56	5.1	11	340	2.5	<LOD	<LOD	<LOD
	Novigrad	51	4.8	5.6	96	2.4	<LOD	<LOD	<LOD
River (WWTP)	Ljubljanica	74	<LOD	3.2	24	<LOD	2900	320	19
	K. Bistrica	190	<LOD	2.9	12	<LOD	21	24	6.6
	Krka	54	<LOD	<LOD	5.9	<LOD	<LOD	<LOD	<LOD

Table 26: Levels (ng g^{-1}) of target benzophenones in sediment samples collected from different surface waters.

Matrix	Sampling Site	BP	H-BP	DH-BP	HM-BP	DHM-BP	KP	EtBP	AcBP
Fluvial sediment	Kolpa	610	<LOD	3.5	32	<LOD	<LOD	<LOD	<LOD
	Krka	650	<LOD	2.6	31	<LOD	<LOD	<LOD	<LOD
	Nadiža	620	<LOD	<LOD	23	<LOD	<LOD	<LOD	<LOD
Lacustrine sediment	Bled	300	<LOD	3.7	17	<LOD	<LOD	<LOD	<LOD
	Šobec	360	<LOD	2.5	17	<LOD	<LOD	<LOD	<LOD
	Rakitna	300	<LOD	<LOD	14	<LOD	<LOD	<LOD	<LOD
	Bohinj	280	<LOD	<LOD	12	<LOD	<LOD	<LOD	<LOD
	Bloke	220	<LOD	<LOD	10	<LOD	<LOD	<LOD	<LOD
Marine sediment	Portorož	270	<LOD	<LOD	13	<LOD	<LOD	<LOD	<LOD
	Ankaran	280	<LOD	<LOD	12	<LOD	<LOD	<LOD	<LOD
	Novigrad	270	<LOD	2.0	10	<LOD	<LOD	<LOD	<LOD
Fluvial sediment (WWTP)	Ljubljanica	190	<LOD	<LOD	7.4	<LOD	<LOD	<LOD	<LOD
	K. Bistrica	85	<LOD	3.1	4.2	<LOD	<LOD	<LOD	<LOD
	Krka	38	<LOD	<LOD	2.5	<LOD	<LOD	<LOD	<LOD

The occurrence of UV filters in surface waters and sediments is clearly affected by recreational activities such as swimming and bathing rather than by WWTP discharges. In contrast, the major source of KP and its transformation products in surface waters are WWTP effluents. Also, the target compounds occur more frequently in water samples, as only three benzophenones (BP, HM-BP and DH-BP) have been detected in sediments. This may be explained by the low total organic carbon (TOC) and total nitrogen (TN) values determined in the samples (Table 27), since organic matter is the most important binding site for organic compounds in sediments. In addition, the physico-chemical properties of the target analytes (see Section 1.1.3) imply that these compounds are more likely to remain in the water phase than to be bound to solid particles. The low levels of the target compounds in sediment samples may also originate from sediment characteristics, since the majority of samples are mostly composed of sand (Table 27) rather than silt and clay, which usually accumulate greater concentrations of contaminants than course particles (Förstner, 2004). Since benzophenones are unlikely to dissociate at environmental conditions (see Section 1.1.3), ion exchange mechanism is not an important binding process for these compounds and cation exchange capacity (CEC) values (Table 27) do not limit their presence in the investigated sediment samples.

Not surprisingly, the most frequently detected compounds in water and sediment samples were BP and HM-BP, as they are two of the most commonly used UV filters (Jeon et al., 2006; Tarazona et al., 2010). They were also in the highest concentrations. Overall, HM-BP is one of the most commonly detected UV filters in surface waters (Balmer et al., 2005; Cuderman & Heath, 2007; Giokas et al., 2004; Rodil & Moeder, 2008a). In addition, DH-BP was found in most of the water samples (up to 130 ng L^{-1}) and in several sediments (up to 3.7 ng g^{-1}). Since DH-BP is a major metabolite formed from HM-BP (Felix, Hall, & S. Brodbelt, 1998; Kunisue et al., 2012) and is also used as a personal care product, its occurrence may originate from different sources and this could account for the high frequency of its detection. H-BP and DHM-BP were less common in aqueous samples and were not detected in sediments (Table 25 and Table 26).

Studies report that the occurrence of UV filters depends on several factors, such as sample location, size of the system and the intensity of recreational activity (Díaz-Cruz & Barceló, 2009; Giokas et al., 2007). This is also evident from the results of this study, where the highest concentrations were found in lakes. For instance, in Lake Šobec, which is a relatively small lake, known for its high bathing activity in summer months, HM-BP was found in concentration of 820 ng L^{-1}. Both the concentration and frequency of UV filters were lower in rivers and seawater than in lacustrine samples. This may result from a higher dilution factor in the seawater and from the continuous flow in rivers. In general, the concentrations of UV filters are similar to literature values, shown in Table 3 (Balmer et al., 2005; Cuderman & Heath, 2007; Giokas et al., 2004; Kameda et al., 2011; Langford & Thomas, 2008; Poiger et al., 2004; Tarazona et al., 2010). However, only the occurrence of HM-BP has been extensively investigated, while the presence of other benzophenone-type UV filters in the aqueous environment are rarely addressed (Jeon et al., 2006; Kameda et al., 2011; Tarazona et al., 2010), making it difficult to compare the results.

The levels of KP and its transformation products EtBP and AcBP were below the LOD in the investigated surface waters (except in the Ljubljanica and Kamniška Bistrica) and in all the sediment samples. This was expected, since most samples were collected in areas of recreational activity. In addition, the KP dissociation constant (pK_a = 4.45) suggests it is dissociated at environmental pH, indicating its higher mobility in the aqueous phase than expected by its log K_{ow} value (log K_{ow} = 2.81), thus it is not likely to bind to sediments. Ketoprofen is also extremely photolabile (Bosca, Miranda, Carganico, & Mauleon, 1994; Kosjek et al., 2011), and once in the environment, it is likely to undergo photodegradation. However, the Ljubljanica, which was sampled near a WWTP outflow (downstream of the plant), revealed a high concentration of KP (≤ 2900 ng L^{-1}). In addition, its transformation products EtBP (320 ng L^{-1}) and AcBP (19 ng L^{-1}) were also detected. The concentration of KP is higher than most values reported for surface waters (Bendz et al., 2005; Tixier et al., 2003; Vieno et al., 2005). The Ljubljanica is the receiving body of a WWTP, which treats wastewater affected by several hospitals, a pharmaceutical factory and the municipality of Ljubljana, which are all possible sources of KP. Ketoprofen, EtBP and AcBP were also detected in Kamniška Bistrica, which receives effluent from a WWTP, but the concentrations were lower than those in Ljubljanica (Table 25). The present study is the first to report the occurrence of EtBP and AcBP in the aqueous environment (Kotnik, Kosjek, Krajnc, & Heath, 2014). It also confirms the photodegradation pathway of KP, which has been demonstrated in lab-scale experiments (Bosca et al., 1994; Kosjek et al., 2011) and also in this study (Section 4.3), suggesting that the same mechanism occurs in the actual environment.

Table 27: Characteristics of sediment samples.

Matrix	Sampling site	Composition (%)			pH	TOC (%)	TN (%)	CEC (mmol/100 g)
		Sand	Silt	Clay				
Fluvial sediment	Kolpa	93.3	3.90	2.80	7.5	0.7	0.1	19.4
	Krka	36.6	43.3	20.1	7.5	5.4	0.3	33.7
	Nadiža	51.4	38.2	10.4	7.6	1.5	0.1	24.3
Lacustrine sediment	Bled	85.6	8.70	5.70	7.2	2.8	0.1	21.2
	Šobec	64.4	31.5	4.10	6.9	3.1	0.3	21.8
	Rakitna	97.5	1.00	1.50	7.4	0.7	0.0	18.5
	Bohinj	92.8	4.20	3.00	7.4	0.9	0.1	19.3
	Bloke	21.1	37.5	41.4	7.3	2.4	0.2	31.1
Marine sediment	Portorož	97.0	1.50	1.50	8.0	0.3	0.0	36.8
	Ankaran	96.0	2.00	2.00	7.8	0.4	0.0	34.1
	Novigrad	93.5	1.50	5.00	8.3	0.9	0.1	153
Fluvial sediment (WWTP)	Ljubljanica	78.7	15.0	6.30	7.5	1.5	0.1	20.7
	K. Bistrica	85.9	10.0	4.10	6.9	8.7	0.5	26.8
	Krka	53.9	28.1	18.0	7.4	6.1	0.3	33.0

4.3 Photodegradation of Benzophenones

Photodegradation of the target benzophenones was investigated using laboratory scale UV irradiation with MP and LP mercury lamps, and by natural sunlight.

4.3.1 UV irradiation

The photolysis parameters of MP/UV irradiation for the investigated benzophenones are presented in Table 28, while the photodegradation process is shown in Figure 25. Where depicted with error bars, experiments were performed in triplicate. Since preliminary experiments showed good repeatability with a RSD < 10% for all benzophenones treated with MP/UV at different exposure times (Table 29), not all the experiments were performed in triplicate.

Photodegradation of benzophenones followed pseudo-first-order kinetics as plots of natural logarithms of concentration in aqueous solution versus time yielded straight lines, described by the following equation:

$$\ln \frac{c_t}{c_0} = -kt \quad (4)$$

where c_t represents the concentration at time t, c_0 is the initial concentration, k is the degradation rate constant and t is the reaction time.
Half-lives were calculated according to Equation 5:

$$t_{1/2} = \frac{ln\,2}{k} \quad (5)$$

The experiment confirmed the low photostability of KP, since 10 minutes of irradiation resulted in 99.3% elimination (Figure 24). The photolabile character of KP has been reported in previous studies using different experimental conditions (Kim et al., 2009; Kosjek et al., 2011; Lin & Reinhard, 2005; Salgado et al., 2013; Szabó et al., 2011). The half-life of KP (Table 28) also corresponds well with reported $t_{1/2}$ values of 0.27 min to 64.2 min (Lin & Reinhard, 2005; Matamoros et al., 2009; Salgado et al., 2013). Irradiation of KP resulted in the formation of EtBP and AcBP (Figure 24). Initially, the formation of

EtBP was more dominant, while AcBP reached a peak concentration after 15 minutes. After 40 minutes, both compounds were hardly detectable, which may be the result of their decreased formation or their subsequent degradation by UV light. Hence, photodegradation of these two compounds was investigated individually (Figure 25). Table 28 shows that EtBP was more prone to UV light, being almost completely decomposed within 60 minutes (degradation constant of 0.041 min^{-1}), while AcBP was more stable with a degradation constant of 0.023 h^{-1}. While EtBP and AcBP are known KP transformation products (Kosjek et al., 2011; Szabó et al., 2011), their photolytic behavior is yet to be investigated.

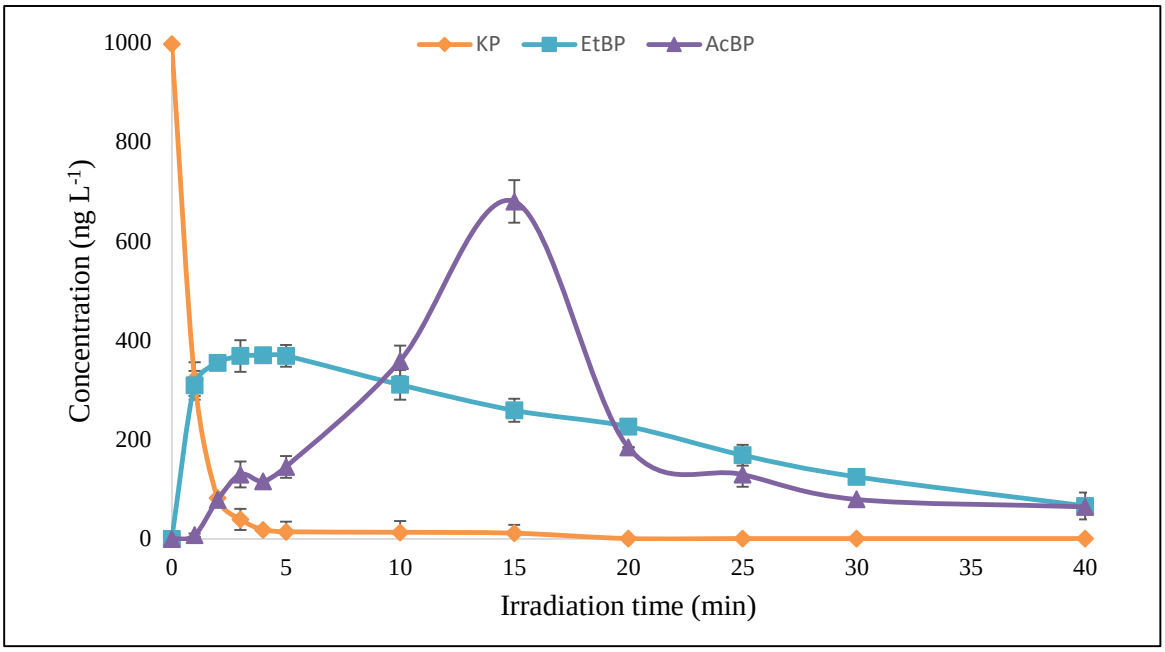


Figure 24: MP/UV irradiation of KP and subsequent formation of its phototransformation products EtBP and AcBP (n = 3).

UV filters are designed to be photostable to guarantee long-term protection of human skin and other products from sunlight. In the present study, 7 h of MP/UV irradiation resulted in the elimination of only ≤ 21% of the parent compounds. However, prolonged irradiation times did show that benzophenones were, to a certain extent, photodegradable. Their degradation followed pseudo-first-order reaction kinetics, resulting in half-lives of 17 h to 99 h (Table 28). The most photostable were DHM-BP and HM-BP. This agrees with previous studies (Gago-Ferrero et al., 2012; Liu et al., 2011; Rodil et al., 2009), where elimination of HM-BP was < 5% after 9 h of irradiation (λ = 254 nm) and 8% when exposed to artificial solar irradiation (290–800 nm) for 50 days (Liu et al., 2011). In contrast, elimination of DH-BP was 62% after 24 h of exposure to MP/UV. Gago-Ferrero et al. (2012) reported similar photolytic behavior for DH-BP, which was completely degraded after 24 h of irradiation by simulated sunlight. HM-BP remained unaltered.

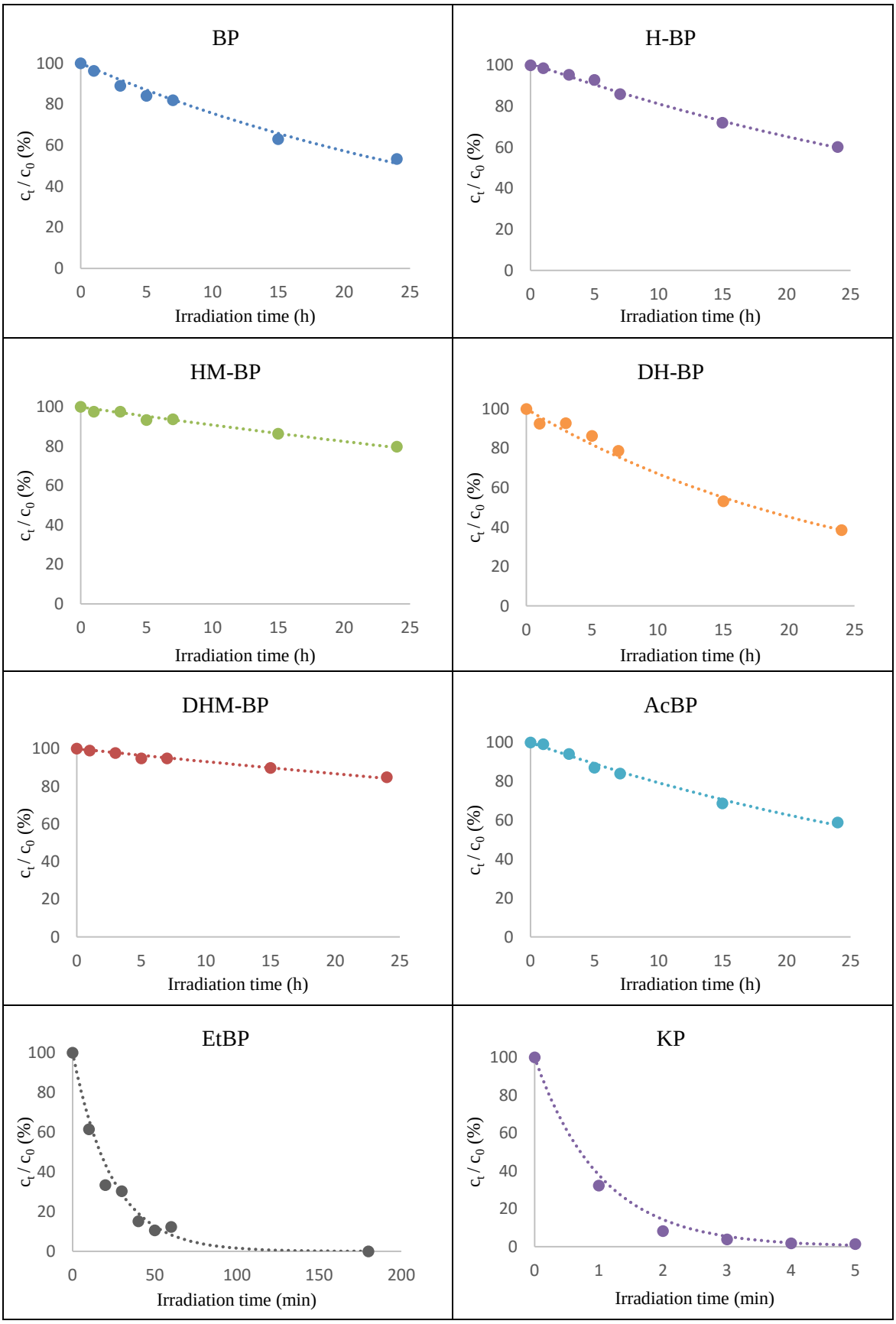


Figure 25: Photolytic behavior of benzophenones exposed to MP/UV irradiation.

Table 28: Photodegradation parameters of MP/UV irradiation of the investigated benzophenones.

Analyte	Irradiation period	*k*	$t_{1/2}$	c_t / c_0 (%)	r^2
BP	0–24 h	0.026 h^{-1}	26.3 h	53.3 (t = 24 h)	0.988
H-BP		0.022 h^{-1}	31.8 h	60.1 (t = 24 h)	0.996
HM-BP		0.009 h^{-1}	76.2 h	79.8 (t = 24 h)	0.986
DH-BP		0.041 h^{-1}	17.0 h	38.4 (t = 24 h)	0.990
DHM-BP		0.007 h^{-1}	99.0 h	84.7 (t = 24 h)	0.988
AcBP		0.023 h^{-1}	30.5 h	58.8 (t = 24 h)	0.990
EtBP	0–180 min	0.041 min^{-1}	17.0 min	0.06 (t = 180 min)	0.992
KP	0–10 min	0.879 min^{-1}	0.79 min	0.72 (t = 10 min)	0.959

Table 29: Relative standard deviations for MP/UV irradiation of benzophenones in distilled water.

Analyte	RSD (%)	
	MP/UV irradiation (n =3)	
	t = 1 h	t = 15 h
BP	3	8
H-BP	1	1
DH-BP	6	6
HM-BP	2	9
DHM-BP	4	1
AcBP	7	8
	t = 10 min	t = 40 min
EtBP	4	6
	t = 1 min	t = 3 min
KP	7	5

Since LP lamps are used in water treatment for disinfection purposes (Liu et al., 2011; Szabó et al., 2011), the behavior of benzophenones exposed to LP/UV light was also investigated. The photolytic parameters as well as a comparison with the MP lamp efficiency are presented in Table 30. The degradation kinetics followed the same order as for the MP/UV irradiation, while irradiation at 254 nm resulted in higher photolysis rates and decreased half-lives. Benzophenones have maximum absorption capacity at a wavelength of around 250 nm, which means they are more susceptible to LP/UV than MP/UV irradiation.

Table 30: Comparison of photolytic parameters of benzophenones upon exposure to MP/UV and LP/UV irradiation.

Analyte	MP/UV			LP/UV		
	$t_{1/2}$	k	c_t / c_0 (%)	$t_{1/2}$	k	c_t / c_0 (%)
BP	26.3 h	0.026 h^{-1}	82.0 (7 h)	4.44	0.156 h^{-1}	34.0 (7 h)
H-BP	31.8 h	0.022 h^{-1}	85.9 (7 h)	17.7	0.039 h^{-1}	75.3 (7 h)
HM-BP	76.2 h	0.009 h^{-1}	93.7 (7 h)	19.6	0.035 h^{-1}	77.1 (7 h)
DH-BP	17.0 h	0.041 h^{-1}	78.7 (7 h)	7.37	0.094 h^{-1}	53.3 (7 h)
DHM-BP	99.0 h	0.007 h^{-1}	94.8 (7 h)	22.3	0.031 h^{-1}	79.6 (7 h)
AcBP	30.5 h	0.023 h^{-1}	84.0 (7 h)	3.84	0.181 h^{-1}	26.2 (7 h)
EtBP	17.0 min	0.041 min^{-1}	0.06 (180 min)	7.12	0.097 min^{-1}	99.9 (180 min)
KP	0.791 min	0.879 min^{-1}	0.72 (10 min)	0.861	0.804 min^{-1}	0.032 (10 min)

4.3.2 Solar irradiation

Table 31 and Figure 28 show the photolytic behavior along with the degradation rate constants and half-lives of the investigated benzophenone derivatives, exposed to natural sunlight, and the influence of different aqueous matrices on their photodegradation as a result of direct and indirect photolysis. Where depicted with error bars, experiments were performed in duplicate. Preliminary experiments (Table 33) revealed good repeatability with RSD < 15% at different irradiation times, therefore experiments were not repeated for each exposure time.

The results of solar irradiation in distilled water, which reflect the direct photolysis of benzophenones, are in agreement with the laboratory-scale MP/UV irradiation experiments (Section 4.3.1), however, the results cannot be directly compared due to the differences in the radiation spectrum, intensity and daily light variations (night–day circle). Ketoprofen was the most photolabile with $t_{1/2}$ of 28.2 min, while its transformation products were less affected by sunlight irradiation (Table 31). The high rates of photodegradation for KP are consistent with previously reported studies, where KP was irradiated with a solar simulator (xenon lamp) resulting in a $t_{1/2}$ in Milli-Q water of 0.54–2.9 min (Lin & Reinhard, 2005; Matamoros et al., 2009). Similar results were reported for KP by sunlight irradiation in Milli-Q water with a $t_{1/2}$ < 15 min (Koumaki et al., 2015).

The photostability of UV filters followed the same order as determined by MP/UV irradiation: DHM-BP > HM-BP > H-BP > BP > DH-BP, suggesting that laboratory-scale experiments using the MP lamp can help in predicting the behavior of these compounds in the natural environment.

Table 31: Photolytic parameters of the investigated compounds under solar irradiation in various aqueous matrices.

Analyte	DISTILLED WATER			LAKE WATER			SEAWATER		
	k (h^{-1})	$t_{1/2}$	r^2	k (h^{-1})	$t_{1/2}$	r^2	k (h^{-1})	$t_{1/2}$	r^2
BP	0.0014	20.7 d	0.991	0.0024	12.2 d	0.989	0.0009	32.4 d	0.998
H-BP	0.0013	21.6 d	0.998	0.0030	9.52 d	0.994	0.0012	24.2 d	0.985
HM-BP	0.0001	30.9 d	0.994	0.0031	9.38 d	0.994	0.0007	43.3 d	0.985
DH-BP	0.0025	11.7 d	0.962	0.0064	4.53 d	0.980	0.0090	3.22 d	0.981
DHM-BP	0.0008	38.9 d	0.990	0.0086	3.35 d	0.964	0.0068	4.28 d	0.987
AcBP	0.0001	39.4 d	0.994	0.0026	11.0 d	0.999	0.0003	95.0 d	0.973
EtBP	0.0450	15.4 h	0.986	0.0486	14.3 h	0.994	0.0659	10.5 h	0.988
KP	1.4702	0.47 h	0.975	1.6174	0.43 h	0.992	1.5110	0.46 h	0.992

Figure 26 and Figure 27 show that KP exposed to natural sunlight also transforms into EtBP and AcBP, which occurred in all investigated matrices: distilled, lake and seawater. This confirms KP as their source when they occur in the environment. Nevertheless, after 10 h of irradiation, both compounds were almost undetectable, indicating they are prone to photodegradation, which was investigated in further experiments (Figure 28). EtBP and AcBP have been previously reported as transformation products of KP, exposed to sunlight in Milli-Q water (Koumaki et al., 2015), where AcBP was considered as the most abundant by-product.

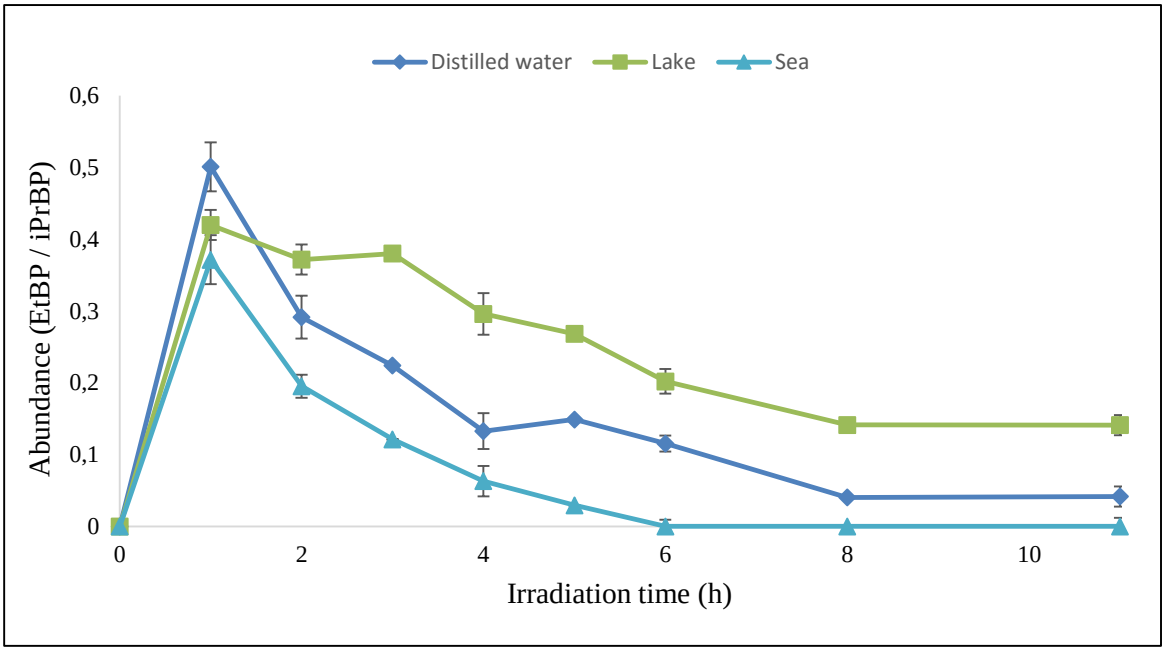


Figure 26: Formation of EtBP upon exposure of KP to sunlight in different aqueous matrices – distilled water, lake and seawater (n = 2).

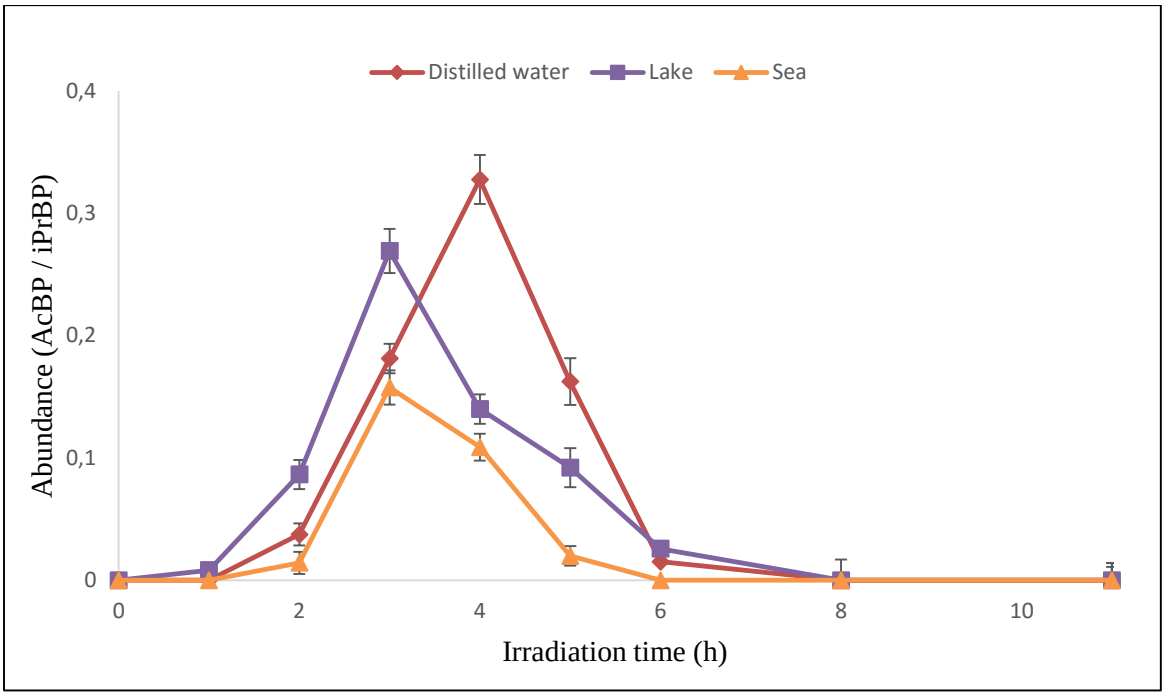


Figure 27: Formation of AcBP upon exposure of KP to sunlight in different aqueous matrices – distilled water, lake and seawater (n = 2).

A comparison of different aqueous matrices showed that indirect photolysis, with the exception of KP, has a significant effect on photodegradation of benzophenones. Lake and seawater were chosen to represent two surface water matrices containing different amounts of DOM and other nutrients, salinity and pH, while distilled water served for comparison to evaluate solely the effect of direct photolysis (Table 32).

The presence of lake water accelerated the photodecomposition of benzophenones, with photolytic half-lives shorter than in distilled water (Table 31). In particular, the half-life of DHM-BP in distilled water was approximately ten times that of lake water (38.9 days and 3.35 days, respectively). Half-lives in seawater, with the exception of DH-BP and DHM-BP, were longer relative to distilled water. The differences in the reaction rates are less evident for EtBP, which has a short environmental half-life in all three matrices. There was no difference in photodegradation rates between distilled water, lake and seawater for KP, which indicates that direct photolysis is the dominant elimination process for this pharmaceutical, since photosensitizers naturally present in waters clearly have no effect on its removal. This has been demonstrated by previous studies, which show no significant difference in photodegradation of KP in Milli-Q water, river water and seawater (Lin & Reinhard, 2005; Matamoros et al., 2009). The determined environmental half-life of KP in surface waters (25.8–27.6 min; Table 31) is in the same range as it has been reported in river water exposed to sunlight ($t_{1/2}$ = 2.4 min) (Matamoros et al., 2009).

The enhanced photodegradation rates of benzophenones in lake water most likely result from the presence of photosensitizers, producing various reactive species during solar irradiation. A comparison of lake and seawater (Table 32) reveals that the most important difference is in the nitrate concentration, which is significantly higher in lake water. Equations 6 and 7 show how solar irradiation of NO_3^- leads to the formation of $NO_2^{\bullet}$ and $^{\bullet}OH$, which can react with the target benzophenones (Koumaki et al., 2015). In contrast, the concentration of nitrate in seawater was considerably lower, and any effect on photolysis would be insignificant. Matamoros et al. (2009), who spiked different concentrations of NO_3^- (0, 1, and 10 mg L^{-1}) in aqueous solutions, previously investigated

the effect of nitrate on the photodegradation of ketoprofen. No difference in the photodegradation of KP was observed at the three concentrations of NO_3^- tested (Matamoros et al., 2009), which is in agreement with the present study.

$$NO_3^- \xrightarrow{h\nu} NO_2^{\bullet} + O^{-\bullet} \quad (6)$$

$$O^{-\bullet} + H_2O \rightarrow OH^{\bullet} + OH^- \quad (7)$$

The concentration of nitrite was slightly higher in seawater than in lake water. Since nitrite is known to be a photosensitizer, this could explain the enhanced photolysis of DH-BP and DHM-BP (Figure 28). TOC and DOC levels were similar in both aqueous matrices (Table 32). The reason for the decreased photodegradation in seawater is because naturally occurring photosensitizers may compete for the light source, acting as a filter hindering light transmission. Although the levels of phosphate and ammonium in seawater were significantly higher than in lake water (Table 32), they are not considered as important environmental photosensitizers (Cao, Lu, & Wang, 2012). Phosphate cannot directly influence photodegradation, however, the intermediates generated in the direct photolysis of the target benzophenones could have reacted with phosphate ions to form new compounds, which further retarded direct photodegradation of benzophenones (Cao et al., 2012). This would also explain the decreased photodegradation of selected compounds in seawater. The pH of lake and seawater is similar to that of distilled water, suggesting that pH is not responsible for the observed differences in the photodegradation rates between the different matrices. This agrees with Koumaki et al. (2015), who found no significant change in the photodegradation rate for KP at pH values from 6 to 8 in Milli-Q water.

The presence of different photosensitizers and scavengers in natural waters means it is difficult to determine which one is responsible for either enhancing or reducing photodegradation in the environment. Nevertheless, from the results it is evident that the photolytic behavior of benzophenones, with the exception of ketoprofen, largely depends on the type of matrix, where these compounds are subjected to direct and indirect photolysis.

Table 32: Surface water parameters measured in photodegradation experiments.

Matrix	pH	TOC (mg L^{-1})	DOC (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (µg L^{-1})	Phosphate (µg L^{-1})	Ammonium (µg L^{-1})
Lake water	7.6	1.63	1.61	5.60	6.90	1.20	< 0.5
Seawater	8.2	1.24	1.18	0.13	8.51	42.3	7.9

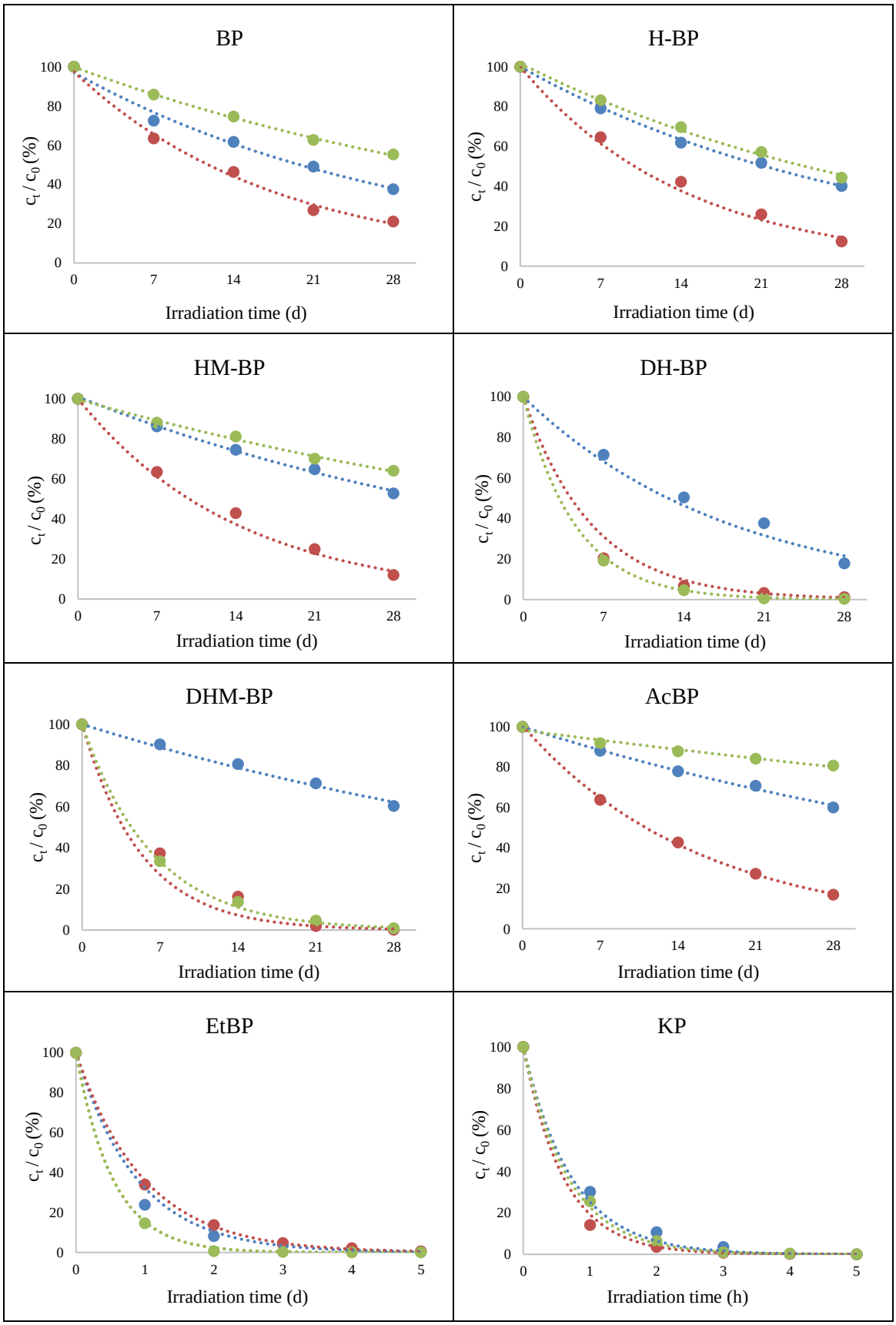


Figure 28: Photolytic behavior of benzophenone derivatives exposed to sunlight in different aqueous matrices: distilled water (●), lake (●) and seawater (●).

Table 33: Relative standard deviations for benzophenones in distilled water exposed to solar irradiation (c_0 = 1 µg L^{-1}).

Analyte	RSD (%)	
	Solar irradiation (n =2)	
	t = 7 d	t = 28 d
BP	10	7
H-BP	9	6
DH-BP	12	11
HM-BP	4	9
DHM-BP	7	11
AcBP	10	13
	t = 1 d	t = 3 d
EtBP	11	5
	t = 1 h	t = 3 h
KP	6	2

4.4 Genotoxicity of Benzophenones

Genotoxicity of benzophenone derivatives was investigated using the SOS/*umuC* assay, which has the advantage of being a fast, simple, inexpensive and sensitive method, convenient for testing a large number of samples (Oda et al., 1985; Reifferscheid et al., 1991). In addition, the assay has been standardized according to ISO (ISO, 2000) for determining the genotoxic potential of native, un-concentrated water samples (Hamer et al., 2000; Žegura et al., 2009).

Genotoxicity of the parent benzophenone derivatives was evaluated by performing the classic SOS/*umuC* assay (Reifferscheid et al., 1991), while the genotoxic potential of the MP/UV photodegradation mixtures was tested following the SOS/*umuC* protocol for unconcentrated water samples, described in the ISO/CD 13829 standard (ISO, 2000), with minor modifications (Žegura et al., 2009).

4.4.1 Genotoxicity of parent compounds

The results of the SOS/*umuC* test are presented in Table 34. The compounds were tested at concentrations between 7.81–1000 µg mL^{-1}, depending on their response and cytotoxicity. All tests were performed both with (+ S9) and without (- S9) metabolic activation.

Ketoprofen and its phototransformation products EtBP and AcBP showed no genotoxic effect in the concentration range tested (Table 34), in neither the presence nor absence of S9. Although AcBP showed cytotoxicity at concentration of 1000 µg mL^{-1} by metabolic activation, bacterial growth was not suppressed by concentrations below 1000 µg mL^{-1}. This study confirmed the results of Philipose et al. (1997), who showed ketoprofen has no mutagenic effect in different *Salmonella* strains using the Ames mutagenicity assay (Philipose et al., 1997). The genotoxicity of EtBP and AcBP is addressed in the present study for the first time (Kotnik, Kosjek, Žegura, Filipič, & Heath, 2016).

Genotoxicity of benzophenone-type UV filters (Table 34) can be compared with the lowest positive effect concentrations (LPEC), introduced by Zhao et al. (2013), using the SOS/*umu* assay. Results show that BP was not genotoxic over the concentration range tested, but when metabolically activated, a concentration of 1000 µg mL^{-1} gave a positive response. Similarly Takemoto et al. (2002) showed the induction of the *umu* gene

expression only after metabolic activation (Takemoto et al., 2002). Zhao et al. (2013) also reported a LPEC value in the same concentration range (LPEC = 769.23 μg mL^{-1}); however, the genotoxic activity was tested only in the absence of metabolic activation. Similar results were observed in this study for H-BP, HM-BP and DH-BP, where all the compounds showed genotoxic activity at ≥ 125 μg mL^{-1} after metabolic activation, while in the absence of S9 no genotoxic effect was observed. All three benzophenones produced a dose dependent genotoxic response. Among them, HM-BP had the highest genotoxic activity, inducing a more than 4-fold increase of β-galactosidase activity (IR = 4.41) at 1000 μg mL^{-1}. H-BP and DH-BP at ≥ 250 μg mL^{-1} were cytotoxic. Zhao et al. (2013) reported similar observations, where H-BP, HM-BP and DH-BP induced genotoxic effects; however, the test was performed only in the absence of metabolic activation. DHM-BP appeared to have no genotoxic activity, in neither the presence nor absence of a metabolic activation system. In general, the genotoxicity of the UV filters can be compared to the values, obtained by Zhao et al. (2013). However, it must be stressed that the concentrations yielding genotoxic activity in both studies were relatively high and are not relevant for environmental exposure.

Table 34: The effect of the investigated compounds on the induction of SOS response in *S. Typhimurium* SOS/*umuC* genotoxicity assay (n = 3).

Analyte	Conc. (μg mL^{-1})	- S9		+ S9	
		G* ± SD	IR** ± SD	G* ± SD	IR** ± SD
BP	62.5	0.96 ± 0.05	1.13 ± 0.092	0.99 ± 0.05	1.56 ± 0.29
	125	0.94 ± 0.03	1.02 ± 0.084	0.99 ± 0.05	1.29 ± 0.39
	250	0.95 ± 0.02	1.10 ± 0.12	0.98 ± 0.02	1.63 ± 0.39
	500	0.91 ± 0.03	1.32 ± 0.051	0.95 ± 0.01	1.78 ± 0.12
	1000	0.74 ± 0.05	1.50 ± 0.067	0.80 ± 0.03	**2.27 ± 0.37**
H-BP	7.81	0.83 ± 0.06	1.64 ± 0.46	0.87 ± 0.08	1.07 ± 0.03
	15.6	0.80 ± 0.04	1.38 ± 0.012	0.81 ± 0.08	1.14 ± 0.13
	31.3	0.78 ± 0.04	1.25 ± 0.44	0.61 ± 0.09	1.37 ± 0.071
	62.5	0.74 ± 0.01	1.30 ± 0.13	0.54 ± 0.08	1.61 ± 0.15
	125	0.65 ± 0.03	1.53 ± 0.011	0.73 ± 0.07	**2.44 ± 0.23**
	250	0.55 ± 0.01	1.77 ± 0.21	0.58 ± 0.04	**2.74 ± 0.32**
	500	**0.46 ± 0.01**	1.95 ± 0.22	0.58 ± 0.30	**2.77 ± 0.67**
	1000	**0.46 ± 0.03**	1.87 ± 0.11	**0.24 ± 0.04**	**5.30 ± 0.67**
HM-BP	62.5	0.98 ± 0.01	1.30 ± 0.063	0.91 ± 0.06	1.88 ± 0.13
	125	0.97 ± 0.02	1.30 ± 0.091	0.95 ± 0.08	1.81 ± 0.19
	250	0.91 ± 0.03	1.35 ± 0.15	0.97 ± 0.04	**2.02 ± 0.17**
	500	0.85 ± 0.01	1.58 ± 0.16	0.84 ± 0.01	**2.62 ± 0.38**
	1000	0.85 ± 0.01	1.39 ± 0.062	0.67 ± 0.08	**4.41 ± 0.89**
DH-BP	7.81	0.92 ± 0.04	1.01 ± 0.054	0.99 ± 0.09	1.04 ± 0.12
	15.6	0.87 ± 0.03	0.977 ± 0.15	0.97 ± 0.10	1.34 ± 0.25
	31.3	0.87 ± 0.03	0.891 ± 0.10	1.00 ± 0.10	1.19 ± 0.15
	62.5	0.83 ± 0.03	0.993 ± 0.10	0.80 ± 0.10	1.72 ± 0.69
	125	0.59 ± 0.04	1.67 ± 0.12	0.64 ± 0.10	**2.74 ± 0.79**
	250	**0.19 ± 0.03**	**2.13 ± 0.40**	0.58 ± 0.08	**2.36 ± 0.80**

	500	**0.02 ± 0.01**	**3.71 ± 2.10**	**0.31 ± 0.04**	**4.27 ± 0.61**
	1000	**0.02 ± 0.01**	**5.13 ± 2.10**	**0.44 ± 0.07**	**4.21 ± 0.14**
DHM-BP	62.5	0.88 ± 0.03	1.13 ± 0.17	0.89 ± 0.20	1.28 ± 0.23
	125	0.89 ± 0.06	0.931 ± 0.042	0.98 ± 0.05	1.22 ± 0.062
	250	0.86 ± 0.05	0.990 ± 0.051	1.00 ± 0.07	1.30 ± 0.32
	500	0.83 ± 0.03	1.12 ± 0.22	1.10 ± 0.10	1.13 ± 0.094
	1000	0.90 ± 0.07	1.20 ± 0.12	1.10 ± 0.20	1.58 ± 0.061
KP	62.5	0.90 ± 0.05	1.18 ± 0.13	1.10 ± 0.06	0.654 ± 0.062
	125	0.92 ± 0.07	1.17 ± 0.043	1.10 ± 0.06	0.812 ± 0.035
	250	0.84 ± 0.06	1.11 ± 0.041	0.91 ± 0.03	0.981 ± 0.14
	500	0.75 ± 0.04	1.27 ± 0.27	0.74 ± 0.04	1.37 ± 0.12
	1000	0.66 ± 0.02	1.48 ± 0.035	0.73 ± 0.10	0.881 ± 0.24
EtBP	62.5	0.99 ± 0.03	0.722 ± 0.14	0.89 ± 0.10	1.12 ± 0.15
	125	0.95 ± 0.05	0.973 ± 0.48	0.83 ± 0.06	1.10 ± 0.10
	250	0.95 ± 0.02	1.03 ± 0.28	0.93 ± 0.08	1.07 ± 0.081
	500	0.86 ± 0.06	1.30 ± 0.13	0.85 ± 0.10	1.10 ± 0.064
	1000	0.83 ± 0.04	1.20 ± 0.19	0.91 ± 0.10	0.981 ± 0.031
AcBP	62.5	0.78 ± 0.03	1.11 ± 0.28	1.00 ± 0.10	0.867 ± 0.12
	125	0.78 ± 0.04	1.53 ± 0.082	0.85 ± 0.20	1.05 ± 0.052
	250	0.72 ± 0.09	1.13 ± 0.21	0.69 ± 0.20	1.33 ± 0.054
	500	0.68 ± 0.01	1.39 ± 0.17	0.68 ± 0.07	1.19 ± 0.051
	1000	0.64 ± 0.03	1.47 ± 0.55	**0.29 ± 0.08**	**2.59 ± 0.15**

* Bacterial growth rate (G): the ratio of the bacterial growth of the water sample treated bacteria to that of the distilled water control

** Induction ratio (IR): the ratio of β-galactosidase activity of the water sample treated bacteria to that of the distilled water control

4.4.2 Genotoxicity of photodegradation mixtures

The genotoxic potential of photodegradation mixtures produced by MP/UV irradiation of benzophenones is presented in Table 35. Most of the degradation mixtures did not elicit genotoxic activity at the concentrations tested, in neither the presence nor absence of a metabolic activation system. The induction ratio increased slightly (IR > 1.5) after BP MP/UV irradiation (IR = 1.61 and IR = 1.52 after 7 h and 25 h of irradiation, respectively), which indicates that the phototransformation products of BP may exhibit weak genotoxic properties. Also, DH-BP showed an elevated induction ratio, which increased with the reduction of the parent compound. However, at given concentration, DH-BP exhibited cytotoxic activity, both in the presence and absence of S9, thus it was not possible to determine the genotoxic potential of DH-BP without affecting the cell growth. For this reason, DH-BP was further irradiated at an initial concentration of 10 mg L^{-1} for 48 h. At this concentration, no inhibition of bacterial growth was observed, but neither did any of the degradation mixtures exhibit genotoxic activity (Table 35).

Given that environmental levels of benzophenones are much lower and different transformation products may be formed at these concentrations, genotoxicity of benzophenones degradation mixtures with the initial concentration of 1 μg L^{-1} was also evaluated. After 24 h of irradiation using the MP lamp, none of the compounds showed any

genotoxic effect, in neither the presence nor absence of a metabolic activation system (Table 35).

Table 35: Genotoxic activity of MP/UV photodegradation mixtures (n = 3).

Analyte	Irradiation time	c_t / c_0 (%)	- S9		+ S9	
			G ± SD	IR ± SD	G ± SD	IR ± SD
MP/UV (c_0 = 100 mg L^{-1})						
BP	0		0.70 ± 0.02	1.12 ± 0.05	0.75 ± 0.05	0.931 ± 0.22
	7 h	88.8	0.73 ± 0.07	**1.61 ± 0.18**	0.93 ± 0.12	1.19 ± 0.12
	24 h	52.6	0.86 ± 0.01	**1.52 ± 0.05**	1.00 ± 0.01	0.971 ± 0.14
H-BP	0		0.67 ± 0.03	1.37 ± 0.17	0.71 ± 0.03	0.968 ± 0.25
	7 h	85.2	0.64 ± 0.01	1.09 ± 0.04	0.75 ± 0.10	0.804 ± 0.17
	24 h	50.8	0.64 ± 0.01	1.02 ± 0.05	0.83 ± 0.02	1.01 ± 0.27
HM-BP	0		0.82 ± 0.04	1.00 ± 0.05	0.97 ± 0.06	1.11 ± 0.022
	7 h	93.2	0.78 ± 0.01	1.02 ± 0.03	0.88 ± 0.03	0.944 ± 0.10
	24 h	81.5	0.78 ± 0.04	1.07 ± 0.12	0.93 ± 0.11	1.01 ± 0.061
DH-BP	0		**0.35 ± 0.06**	**1.71 ± 0.09**	0.69 ± 0.07	1.29 ± 0.19
	7 h	87.2	**0.22 ± 0.01**	**1.74 ± 0.14**	0.56 ± 0.05	1.14 ± 0.11
	24 h	56.2	**0.13 ± 0.01**	**2.81 ± 0.63**	**0.44 ± 0.04**	**1.52 ± 0.27**
DHM-BP	0		0.53 ± 0.02	1.32 ± 0.03	0.84 ± 0.05	1.13 ± 0.031
	7 h	92.0	0.79 ± 0.03	1.24 ± 0.09	0.87 ± 0.01	1.04 ± 0.233
	24 h	79.6	0.83 ± 0.03	1.05 ± 0.07	0.92 ± 0.02	1.06 ± 0.052
KP	0		0.90 ± 0.03	1.09 ± 0.05	0.91 ± 0.10	1.16 ± 0.091
	5 min	1.71	0.70 ± 0.04	1.48 ± 0.11	0.89 ± 0.02	1.10 ± 0.13
	10 min	0.56	0.74 ± 0.04	1.36 ± 0.13	0.92 ± 0.08	1.29 ± 0.14
MP/UV (c_0 = 10 mg L^{-1})						
DH-BP	0		0.87 ± 0.07	1.24 ± 0.09	0.98 ± 0.09	1.01 ± 0.014
	7 h	84.5	0.91 ± 0.05	1.26 ± 0.10	1.00 ± 0.19	1.08 ± 0.21
	24 h	51.1	0.94 ± 0.11	1.19 ± 0.08	1.10 ± 0.10	1.21 ± 0.13
	48 h	40.8	0.97 ± 0.07	1.18 ± 0.08	1.10 ± 0.10	1.09 ± 0.14
MP/UV (c_0 = 1 µg L^{-1})						
BP	0		1.20 ± 0.18	1.09 ± 0.19	0.75 ± 0.10	1.18 ± 0.083
	24 h	53.3	1.10 ± 0.04	0.861 ± 0.08	1.00 ± 0.20	0.834 ± 0.80
H-BP	0		1.10 ± 0.03	0.921 ± 0.13	1.00 ± 0.20	0.842 ± 0.10
	24 h	60.1	1.20 ± 0.08	0.974 ± 0.13	0.99 ± 0.27	0.901 ± 0.10
HM-BP	0		1.10 ± 0.12	0.775 ± 0.09	1.10 ± 0.39	1.05 ± 0.11
	24 h	79.8	1.10 ± 0.03	0.779 ± 0.08	1.00 ± 0.24	0.921 ± 0.12
DH-BP	0		1.10 ± 0.06	0.807 ± 0.14	1.10 ± 0.18	0.854 ± 0.11
	24 h	38.4	1.10 ± 0.09	0.746 ± 0.16	1.00 ± 0.06	0.892 ± 0.15
DHM-BP	0		1.10 ± 0.13	0.621 ± 0.17	1.00 ± 0.08	1.00 ± 0.11
	24 h	84.7	1.10 ± 0.06	0.763 ± 0.14	0.90 ± 0.14	1.07 ± 0.073
KP	0		1.00 ± 0.06	0.852 ± 0.07	0.90 ± 0.19	0.983 ± 0.10
	10 min	1.11	1.10 ± 0.08	1.04 ± 0.12	1.00 ± 0.35	1.00 ± 0.10

* Bacterial growth rate (G): the ratio of the bacterial growth of the water sample treated bacteria to that of the distilled water control

** Induction ratio (IR): the ratio of β-galactosidase activity of the water sample treated bacteria to that of the distilled water control

5 Conclusions

The present research addresses concerns regarding benzophenone derivatives in the aqueous environment and their potential harmful effects. It provides an insight into surface water pollution and gives a better understanding of the cycling, fate and toxicity of benzophenone derivatives in the aquatic environment.

The developed analytical method for the trace level determination of target benzophenones in environmental samples revealed that solid phase extraction of aqueous samples, and microwave assisted extraction of sediment samples, offer optimal analyte extraction efficiencies. MAE yielded up to 50% higher extraction efficiencies from sediments than UAE, confirming hypothesis H1 stating MAE is more efficient in extracting benzophenones from sediments than UAE. In addition, the present study is the first to address extraction of benzophenones, with the exception of ketoprofen, from sediment samples by MAE. The developed analytical method allowed the detection and quantification of trace amounts of benzophenone derivatives in surface waters and sediments with overall LODs of $\leq$ 1.9 ng L^{-1} for water samples and $\leq$ 1.4 ng g^{-1} for sediments.

Application of the developed method to environmental samples enabled the assessment of benzophenones in both water and sediment samples, which is important since most studies have failed to address their presence in sediments. Unlike grab aqueous samples, sediment samples are much less sensitive to environmental changes and thus provide information about historical pollution. Hypothesis H2, stating that benzophenone-based compounds are present in the Slovenian aquatic environment, was confirmed, since the target benzophenones were detected in both aqueous and sediment samples, sampled across Slovenia. The contamination of surface waters is comparable with published data for European waters. UV filters were present in the majority of the surface waters in concentrations up to 820 ng L^{-1}. In contrast, ketoprofen ($\leq$ 2900 ng L^{-1}) and its transformation products EtBP and AcBP were detected in only two rivers, both receiving WWTP effluents. These results indicate that direct input into bathing waters contributes significantly to UV filter contamination of surface waters, while WWTP effluents are a significant source of ketoprofen pollution. In addition, the study showed that the occurrence of benzophenone-type UV filters in surface waters depends on the size of the water body, as well as the intensity of recreational activity, with the highest concentration found in a relatively small Lake Šobec, which is known for its high bathing activity. Sediment analysis revealed benzophenone to be present in concentrations of up to 650 ng g^{-1}, while concentrations of other compounds were considerably lower, which confirms the low/moderate ability of benzophenones to adsorb to sediments. Importantly, the newly developed method has allowed for the first time the presence of ketoprofen transformation products EtBP and AcBP in the aqueous environment to be reported.

The study shows photodegradation is a significant elimination process for ketoprofen. In addition, UV filters, which are designed to be photostable, were shown to degrade after prolonged irradiation times. The study revealed that EtBP and AcBP are actually formed in the environment by exposure of KP to sunlight. This is the first report of their photolytic behavior under the influence of UV and natural sunlight, where it was shown they also photodegrade into other transformation products, whose fate and effects remain unclear. In

contrast, UV filters are more persistent and due to their semi-polar character have the potential to accumulate in living organisms, which merits further consideration.

Moreover, hypothesis H3, which stated that the type of aqueous matrix influences the photolytic behavior of selected benzophenones, was confirmed by showing that the photolysis of benzophenones depends largely on the matrix type, resulting in both inhibited and enhanced degradation. The exception is ketoprofen, which showed the same photolytic behavior in lake and seawater as in distilled water, implying direct photodegradation is the dominant process. Other benzophenones revealed different photolysis rates in surface waters than in distilled water, indicating they have the potential to undergo direct and indirect photolysis. To assess the environmental half-lives of these compounds, it is important to perform experiments under conditions as close to the natural environment as possible. Nonetheless, the study demonstrated that MP/UV light source can serve as a tool to evaluate the photolytic potential of selected compounds in terms of predicting their environmental fate. This confirms the hypothesis H4, stating that UV laboratory experiments can serve as the basis for assessment of the environmental fate of selected benzophenones under the influence of solar irradiation.

Evaluation of genotoxicity of selected benzophenones using the SOS/*umuC* assay revealed benzophenone derivatives have different genotoxic potential. When metabolically activated, H-BP, HM-BP and DH-BP showed genotoxic activity at ≥ 125 μg mL^{-1}, while BP was genotoxic at concentrations ≥ 1000 μg mL^{-1}. This confirms hypothesis H5, stating benzophenone derivatives have genotoxic potential. However, the hypothesis was not confirmed in terms of ketoprofen, its phototransformation products EtBP and AcBP, and UV filter DHM-BP, which showed no genotoxic potential tested at experimental conditions used in study. Nevertheless, genotoxicity of benzophenones should be further tested. In addition to bacterial models, eukaryotic organisms should be used to evaluate genotoxic activity of these compounds, and especially longer exposure times and low doses should be tested to mimic environmental conditions relevant for humane exposure. The present research is the first to investigate genotoxicity of EtBP and AcBP. In addition, this is the first report of genotoxicity assessment of benzophenones photodegradation mixtures, which revealed no significant increase in genotoxicity after UV irradiation. However, it remains important to evaluate both chemical and toxicological data to obtain a deeper insight into potentially harmful effects of the benzophenones for a more reliable risk assessment.

In conclusion, the present thesis provides new information regarding the presence of benzophenone-derived compounds in the aquatic environment, their behavior, fate and toxicity. The aims set for this study were successfully achieved and its hypotheses confirmed. Despite this, many gaps in the knowledge remain and any future work should involve a more comprehensive survey of the extent of contamination in natural waters as well as focusing on their transport and fate including photodegradation, biodegradation, and toxicity studies. These toxicity studies should involve, in addition to genotoxicity, also other toxicity tests such as estrogenicity and ecotoxicity, and investigating toxicity of mixtures of compounds. Transformation products, formed by the exposure of benzophenones to different environmental conditions, should also be identified and investigated. The combined results of such a study will provide information on the threat posed by benzophenone-derived compounds in the environment.

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Appendix

Kristina Kotnik (formerly Kristina Pestotnik)

Personal bibliography for the period 2010–2016

Article - component part

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1. Kotnik, K., Kosjek, T., Žegura, B., Filipič, M., & Heath, E. (2016). Photolytic fate and genotoxicity of benzophenone-derived compounds and their photodegradation mixtures in the aqueous environment. *Chemosphere*, 147, 114-123. doi:http://dx.doi.org/10.1016/j.chemosphere.2015.12.068 [COBISS.SI-ID 29138983]

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3. Kotnik (Pestotnik), K., Kosjek, T., & Heath, E. (2014). Transformation Products of Personal Care Products: UV Filters Case Studies. In D. A. Lambropoulou & L. M. Nollet (Eds.), *Transformation Products* of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks (1st ed., Vol. 2, pp. 471-504): Wiley. [COBISS.SI-ID 27560743]

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5. KOTNIK, Kristina, KOSJEK, Tina, KRAJNC, Uroš, HEATH, Ester. Behaviour of benzophenones under the influence of natural sunlight. V: 5. študentska konferenca Mednarodne podiplomske šole Jožefa Stefana = 5th Jožef Stefan International Postgraduate School Students Conference, 23. maj 2013, Ljubljana, Slovenija. TRDIN, Nejc (ur.), et al. *Zbornik = Proceedings*. Ljubljana: Mednarodna podiplomska šola Jožefa Stefana, 2013, str. 50-59. [COBISS.SI-ID 26655783]

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