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ANALYTICAL METHODS BASED ON GAS CHROMATOGRAPHY
MASS SPECTROMETRY FOR THE ANALYSIS OF EMERGING
CONTAMINANTS: UV FILTERS, BISPHENOLS, AND
MICROPLASTICS

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ABSTRACT

This thesis presented the development and optimization of advanced analytical methods including, gas chromatography-mass spectrometry (GC-MS), GC-MS/MS, and Pyrolysis-GC-MS (Py-GC-MS) for the detection and quantification of emerging contaminants: UV filters, bisphenols, and microplastics in complex environmental waters, and *in vitro* models. Given the growing environmental concerns and their human exposure associated with these contaminants, particularly their persistence and impact on ecosystems and human health, this study employed cutting edge, solid phase extraction and solid phase microextraction techniques and chromatographic methods to address these challenges.

The thesis structured into three main topics, each dedicated to a specific class of emerging contaminants. The first topic explored UV filters, specifically their detection and quantification by SPE and GC-MS/MS in Adriatic seawaters, providing insights into their environmental distribution and concentration. Among UV filters, the thesis further investigated the isomerization product of EHMC (ethylhexyl methoxycinnamate), a commonly used UV filter, in water, evaluating its phototransformation process under natural and simulated conditions by HS-SPME and GC-MS. *In vitro* studies on skin models and environmental water matrices, further illustrated the behavior of these compounds in different contexts, contributing to the understanding of their potential risks. The second topic focused on bisphenol A (BPA) and its analogues, which are widely used in plastics and consumer products. The research investigated the environmental fate and persistence of these compounds. An organic-solventless procedure using *in-vial* acetylation SPME was developed and employed to enhance the detection and quantification of bisphenol in various environmental waters. This method allows for improved sensitivity and precision in monitoring bisphenols at trace levels in complex environmental matrices, providing valuable data on their persistence and potential risks to aquatic ecosystems.

Thirdly, analytical pyrolysis (Py-GC-MS) was applied to estimate the presence of added polyethylene and polystyrene in an *in vitro* human colon model system during the duration of the fermentation experiment. A sample pretreatment was adopted based on Fenton oxidation. The results confirmed the presence of microplastics supporting the observed biological effects. Meanwhile, they highlighted the challenges of detecting microplastics in a complex organic matrix.

This research contributes significantly to the development of optimized analytical methodologies for emerging contaminants, enhancing our ability to monitor UV filters, bisphenols, and microplastics. The findings underscore the need for effective environmental monitoring and regulation to mitigate the risks associated with these pervasive and persistent contaminants.

I. Introduction

1. UV Filters

1.1. Introduction to UV filters

Skin cancer incidence has gradually increased over the last decade, making it an alarming public-health problem (Nanz et al., 2024; Okobi et al., 2024). In response, international health authorities advise limiting exposure to ultraviolet (UV) radiation by avoiding midday sun, wearing protective clothing, and applying sunscreens. Properly formulated sunscreen products are an effective means to mitigate UV-induced skin damage (Gilbert et al., 2013). These cosmetics have been used for decades and contain ultraviolet (UV) filters—compounds that absorb incident UV radiation and thereby shield the skin from harmful wavelengths of sunlight (Giokas et al., 2007).

Solar ultraviolet that reaches the Earth's surface consists mainly of UVA (320–400 nm) and UVB (~290–320 nm), because UVC (< 280 nm) is effectively filtered by stratospheric ozone. UVA contributes to immediate/persistent pigmentation and photoaging and is commonly divided into UVA2 (320–340 nm) and UVA1 (340–400 nm). UVB also induces pigmentation but, owing to its shorter wavelengths and higher photon energy, is the primary driver of sunburn and erythema (Lorigo et al., 2018; Manaia et al., 2013) (See Figure 1.1).

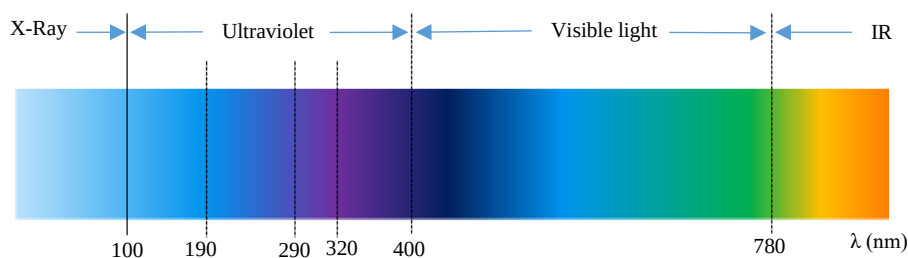


Figure 1.1. *Electromagnetic spectrum*

In sunscreen formulations, UV filters adsorb incident photons and are promoted to excited states before returning to the ground states. Deactivation can occur through photophysical routes (radiative or non-radiative dissipation), through photochemical reactions, or both. Unlike purely photophysical relaxation, photochemical pathways generate transformation products that may be less UV-absorbing (reducing protective efficacy) or may interact with skin biomolecules, other filters, or excipients, potentially provoking photosensitivity (Duarte et al., 2019).

A limited amount of UVC radiation penetrates the ozone layer and reaches the Earth's surface, resulting in immediate erythema and DNA damage upon exposure. This substance is particularly bothersome to the conjunctiva and cornea of the eyes. UVC radiation is utilized in artificial germicidal lamps for the sterilization of medical instruments and environments (Nieradko-Iwanicka & Wysokińska, 2022). The Sun Protection Factor (SPF) of sunscreens indicates their level of protection, primarily against UVB and short-wavelength UVA (UVA2), which are more likely to cause erythema. The term "critical wavelength" refers to the wavelength at which 90% of the 290-400 nm radiation is absorbed; to be considered broad-spectrum, the US FDA requires that sunscreens possess a critical wavelength of no less than 370 nm (Diffey et al., 2000; Ziglar et al., 2025).

UV filters (UVFs) are also incorporated into various consumer products, such as makeup, nail polish, and shampoo, as well as in industrial applications, including plastics, paints, and sealants, to provide protection against photodegradation (Sabzevari et al., 2021).

1.2. Classification and Type of UV filters

UV filters can be categorized into two main types: chemical (organic) filters and physical (inorganic) filters, depending on how they function (See Figure 1.2). Commercial sunscreen formulations generally consist of a blend of organic and inorganic filters, frequently including 20 or more ingredients, to provide comprehensive protection across a wide spectrum (Danovaro et al., 2008; Watkins & Sallach, 2021).

1.2.1. Chemical (Organic) UV filters

Organic UV-filters absorb a UV photon the excess energy is then released as a minimal amount of heat, preventing radiation from directly affecting the skin (Nash & Tanner, 2014). Organic filters can be grouped based on their chemical derivatives into several categories, including *para*-aminobenzoic acid esters, salicylic acid derivatives, cinnamic acid derivatives, benzylidene camphor derivatives, dibenzoyl methane derivatives, benzimidazole and benzotriazole derivatives, triazine derivatives, among others. These filters are further classified as either UVA or UVB filters based on the type of radiation they absorb (Chisvert & Salvador, 2007).

Organic UV filters effectively absorb high-energy UV radiation, transforming it into harmless wavelengths of heat or light that are subsequently released from the skin. These

filters generally comprise chromophores featuring conjugated Π -bond systems, frequently manifested as an aromatic ring linked to a carbonyl ring or connected through a double bond of carbon and carbon (Pniewska & Kalinowska-Lis, 2024). The conjugated electrons are at their lowest energy levels in their ground state. These electrons transition to an excited state upon absorbing an adequate amount of energy, resulting in a transient polarization of the molecule. The excited molecule subsequently returns to its ground state, discharging energy in the form of heat or longer-wavelength radiation (Breakell et al., 2024).

1.2.2. Physical (Inorganic) UV filters

The United States Food and Drug Administration (FDA) has authorized two inorganic (mineral) UV filters: titanium dioxide (TiO_2) and zinc oxide (ZnO), which are both metal oxide particles (Osmond & Mccall, 2010). These inorganic filters interact with UV radiation through two mechanisms: absorption and reflection/scattering. As semiconductor materials, both TiO_2 and ZnO absorb light. They offer protection against both UVA and UVB radiation. In fact, inorganic UV filters can absorb approximately 85 to 95 percent of UV radiation (Cole et al., 2016; Schneider & Lim, 2019). Typically, ZnO particles range in size from 200-400 nm, while TiO_2 particles are between 150-300 nm. The larger particle size is responsible for the white, chalky residue left on the skin, as these particles reflect visible light, which is perceived as white by the human eye. Despite the fact that micronized varieties of titanium dioxide and zinc oxide have been utilized for years, nano-sized versions are now more commonly employed. This innovation enhances the reflection/scattering efficiency while reducing the visible white residue on the skin (Singh & Sharma, 2014).

Similar to organic UV filters, UV radiation excites electrons into higher energy states. The crystalline structure in inorganic filters exhibits overlapping electron orbitals that form “bands,” separated by energy gaps known as the “band gap.” Electrons transition across this gap from a lower valence band to a higher conduction band. The size and composition of particles influence the extent to which UV radiation is absorbed, reflected, or scattered. Nanoscale particles, due to their diminutive size, reflect a reduced amount of visible light, resulting in a nearly transparent appearance, unlike the opaque white of larger particles. Furthermore, decreasing particle size can modify the UV wavelength range in which the particles exhibit optimal effectiveness (National Academies of Sciences & Medicine, 2022).

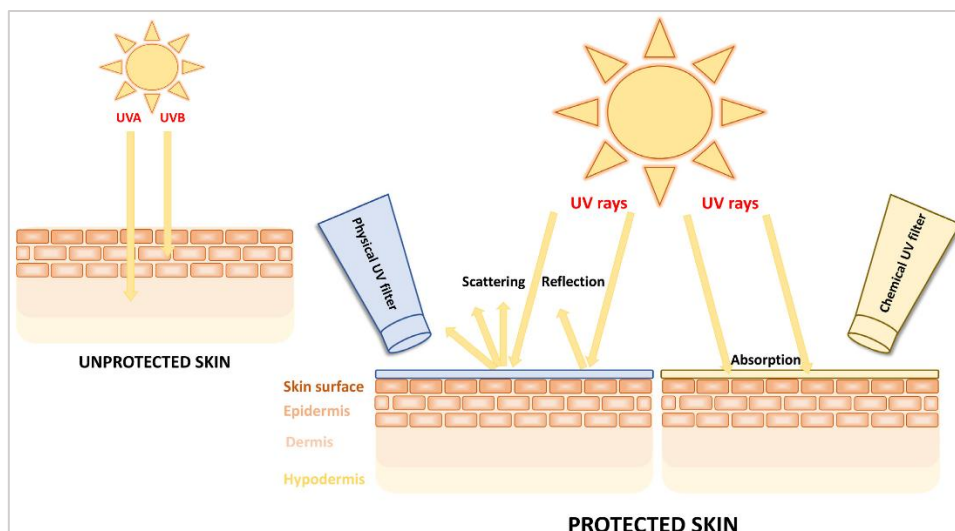


Figure 1.2. Pathways of UV attenuation by physical (inorganic) and chemical (organic) sunscreen agents (Milutinov et al., 2024).

1.3. Structure and Physico-Chemical Properties

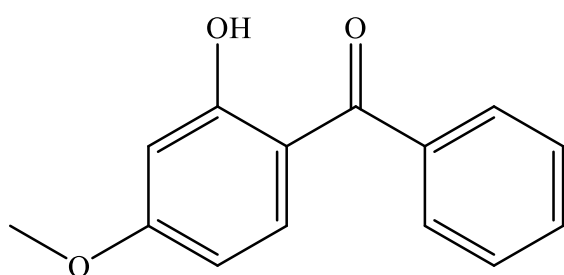
Table 1.1 displays the UV filters used for this investigation, including their identifiers: INCI (International Nomenclature of Cosmetic Ingredients) name, common abbreviation, molecular formula, molecular weight, CAS number, and physicochemical attributes. The parameters include boiling point ($^{\circ}\text{C}$), vapor pressure (mTorr), acidity constant expressed as pK_a ($-\log_{10} K_a$), the decimal logarithm of the octanol-water partition coefficient ($\log K_{ow}$), molar solubility in water, and the wavelength of maximum absorbance. Data were aggregated from ChemSpider (with anticipated values created using the US Environmental Protection Agency's EPISuiteTM) and from SciFinder (calculations performed using ACD/Labs Software v11.02), as shown in Table 1.1.

The compounds generally include one or more aromatic rings, often including conjugated $\text{C}=\text{C}$ bonds and/or carbonyl groups. Most organic UV filters exhibit lipophilicity, rendering them soluble in lipid matrices. The inclusion of ionizable groups, such as carboxylic acid ($-\text{COOH}$) or sulfonic acid (SO_3H), may improve their solubility in water at certain pH levels. (Chisvert & Salvador, 2018). The corresponding chemical structures, created using ChemDraw 20.1.1, are shown in the Figure 1.3.

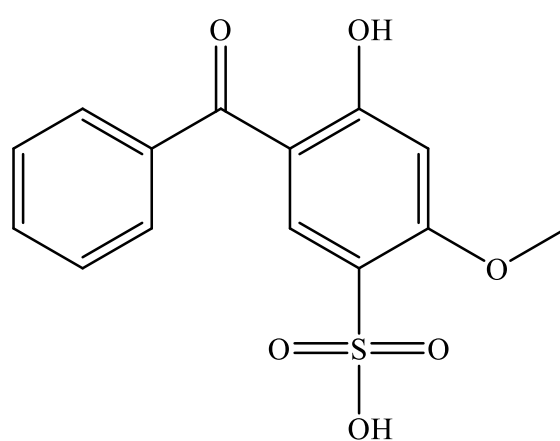
Table 1.1: INCI name, Molecular formula, molecular weight, CAS No. and some physico-chemical properties of studies UV filters.									
INCI name	Molecular Formula	MW (g mol ⁻¹)	CAS No.	Boiling Point (°C)	VP (mTorr)	pK _a	Log k _{ow}	Solubility in water (mol L ⁻¹)	λ max (nm)
Benzophenone-3 (BP3)	C ₁₄ H ₁₂ O ₃	228.24	131-57-7	370±27	0.00526	7.56±0.4	4.0±0.4	pH 1-4: 4.4 x 10 ⁻⁴ pH 7: 5.7 x 10 ⁻⁴ pH 10: 0.10	286, 325
Benzophenone-4 (BP4)	C ₁₄ H ₁₂ O ₆ S	308.31	4065-45-6	498	1.34x10-8	-0.7±0.5	0.9±0.4	pH 1: 2.72 pH 2-10: 3.24	288, 366
2-Ethylhexyl methoxycinnamate (2EHMC)	C ₁₈ H ₂₆ O ₃	290.40	5466-77-3	405±20	8.89x10-4	-	5.9±0.5	2.2 x 10 ⁻⁵	311
Isoamyl methoxycinnamate (IAMC)	C ₁₅ H ₂₀ O ₃	248.32	71617-10-2	363±17	0.0189	-	4.5±0.2	2.4 x 10 ⁻⁴	308

Continued

Table 1.1: INCI name, Molecular formula, molecular weight, CAS No. and some physico-chemical properties of studies UV filters.									
INCI name	Molecular Formula	MW (g mol ⁻¹)	CAS No.	Boiling Point (°C)	VP (mTorr)	pK _a	Log k _{ow}	Solubility in water (mol L ⁻¹)	λ max (nm)
Octocrylene (OCR)	C ₂₄ H ₂₇ NO ₂	361.48	6197-30-4	479±33	2.56 x10 ⁻⁶	-	6.9±0.3	1.0 x 10 ⁻⁶	303
4-Methylbenzylidene camphor (4MBC)	C ₁₈ H ₂₂ O	254.37	36861-47-9	3712±22	9.99 x10 ⁻³	-	3.4±0.3	6.5 x 10 ⁻⁵	300
Ethylhexyl dimethyl PABA (EHPABA)	C ₁₇ H ₂₇ NO ₂	274.30	21245-02-3	383±25	0.00457	2.4±0.1	5.4±0.2	pH 1: 4.1 x 10 ⁻⁴ pH 5-10: 1.7 x 10 ⁻⁵	311
Menthyl anthranilate (MA)	C ₈ H ₉ NO ₂	275.39	134-09-8	384±15	4.38 x10 ⁻³	2.2±0.1	6.1±0.3	pH 1: 4.1 x 10 ⁻⁴ pH 7: 6.6 x 10 ⁻⁵ pH 4-10: 1.3 x 10 ⁻⁵	366

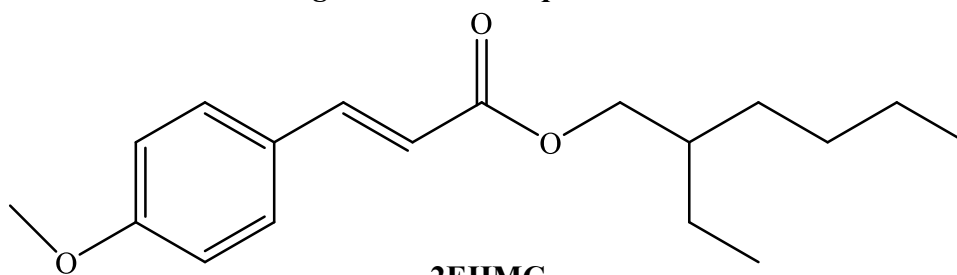


BP-3

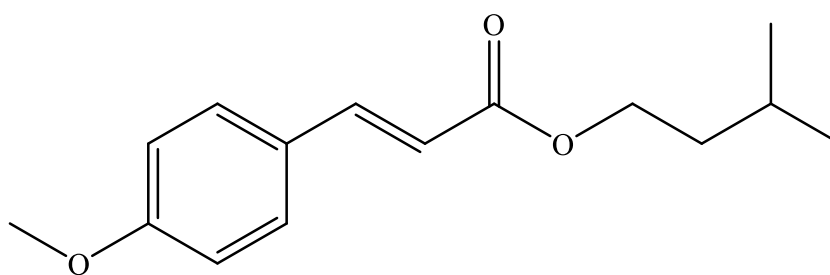


BP-4

Figure 1.3.1. Benzophenones

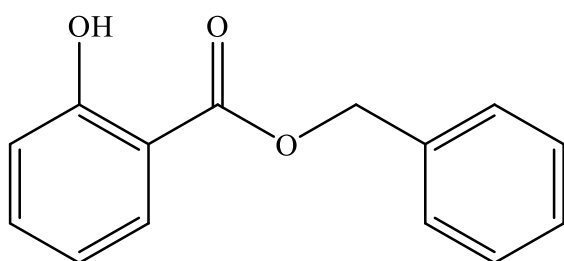


2EHMC

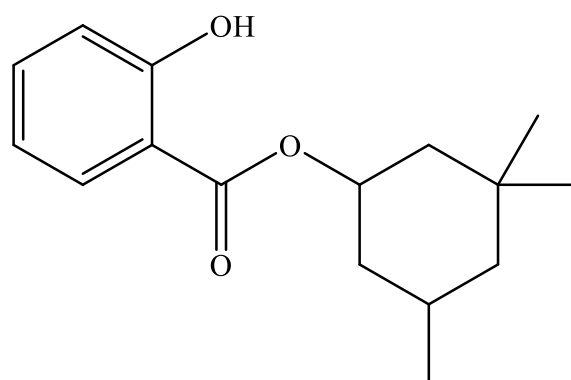


IAMC

Figure 1.3.2. Cinnamates



BS



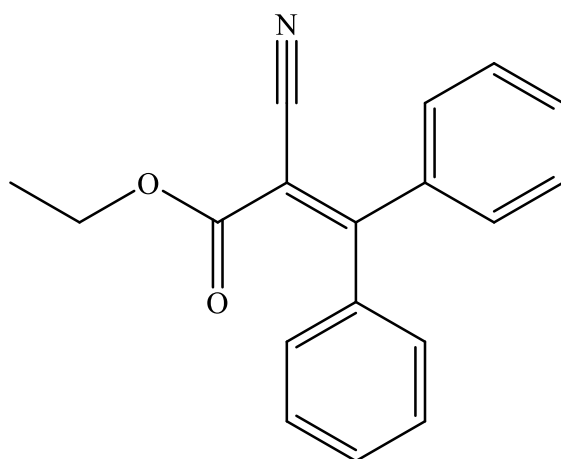
HMS



4MBC

MA





Eto

Figure 1.3.4. Others

1.4.Toxicity and environmental impact

Daily use of UV filters by large populations leads to their entry into aquatic systems through multiple pathways. Dominant inputs include direct sunscreen wash-off during recreation and releases via municipal wastewater, with additional contributions from the degradation or leaching of industrial and consumer materials. In recreational contexts, UV filters are introduced by immediate rinse-off during swimming, aerosol overspray settling, beach run-off, stormwater transport, and effluent from wastewater treatment plants (Mitchellmore et al., 2021; Mitchellmore et al., 2019; National Academies of Sciences & Medicine, 2022; O'Malley et al., 2020; Tovar-Sánchez et al., 2013) (Figure 1.4). Because these compounds originate from diverse matrices, attributing environmental detections to a single source is difficult. Moreover, sunscreens are not the only personal-care source: over 30% of skincare products and about 50% of makeup items contain at least one UV filter (Couteau et al., 2024).

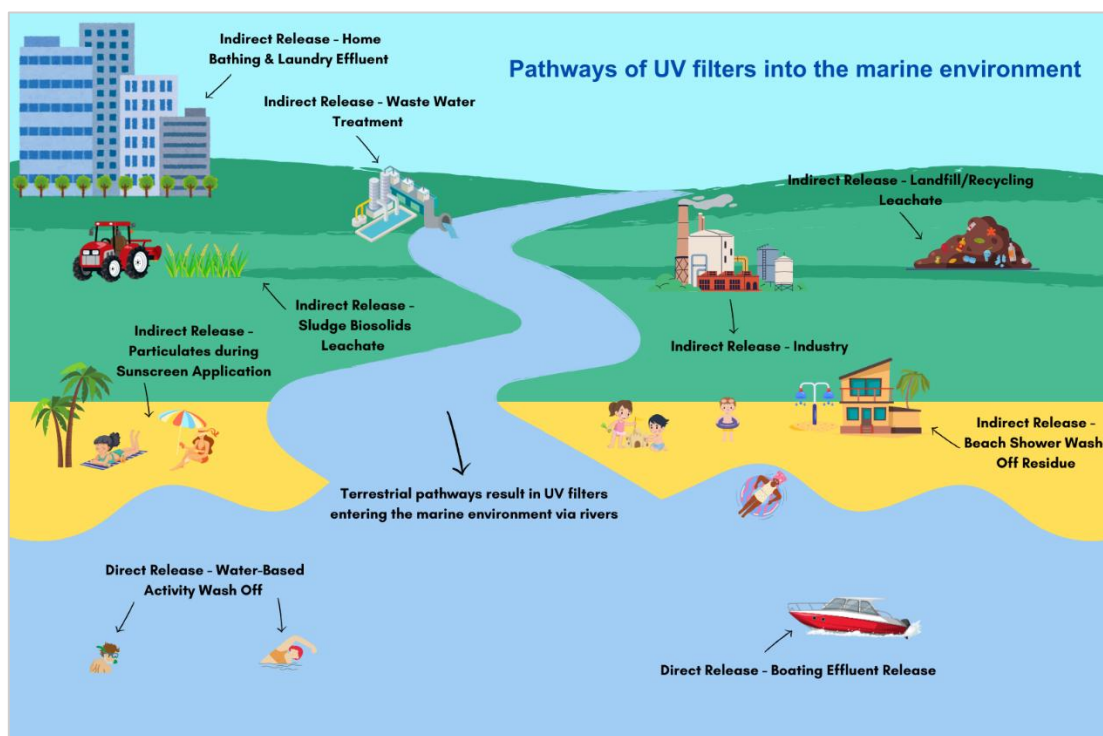


Figure 1.4. Overview of terrestrial and aquatic pathways both direct and indirect transporting UV filters to the marine environments (Hodge et al., 2025).

Many UV filters are not fully biodegradable, and conventional wastewater treatment plants often do not remove them efficiently. Consequently, the environmental loads of these compounds are rising. Multiple UV filters species have been reported in soils, inland

and marine waters, and across diverse biota-including algae, corals, fish, mammals, and terrestrial birds (Couselo-Rodríguez et al., 2022). After entering marine systems via direct or indirect inputs, their fate and ecological impact are governed by physicochemical traits and degradability. Because most UV filters are hydrophobic and only sparingly soluble, they typically occur at low concentrations in the open water column [32]. Instead, they tend to partition into the sea-surface microlayer (the ~0-100 µm air-water interface) and, even more markedly, into marine sediments (Agawin et al., 2024).

To assess how much sunscreens contribute to environmental UV filter loads, it helps to consider the annual production and use of these compounds across personal-care applications. FDA estimates for 2016 (Figure 1.5) indicate that the five most widely used UV filters-OCR, HMS, avobenzone (AVO), octisalate (OCT), and oxybenzone each exceeded roughly 900 million ounces (> 26.6 million kg) of product use. By contrast, quantifying releases from non-sunscreen sources remains difficult because these filters occur in many product categories with heterogeneous usage and disposal patterns (U.S. Food & Drug administration, 2019).

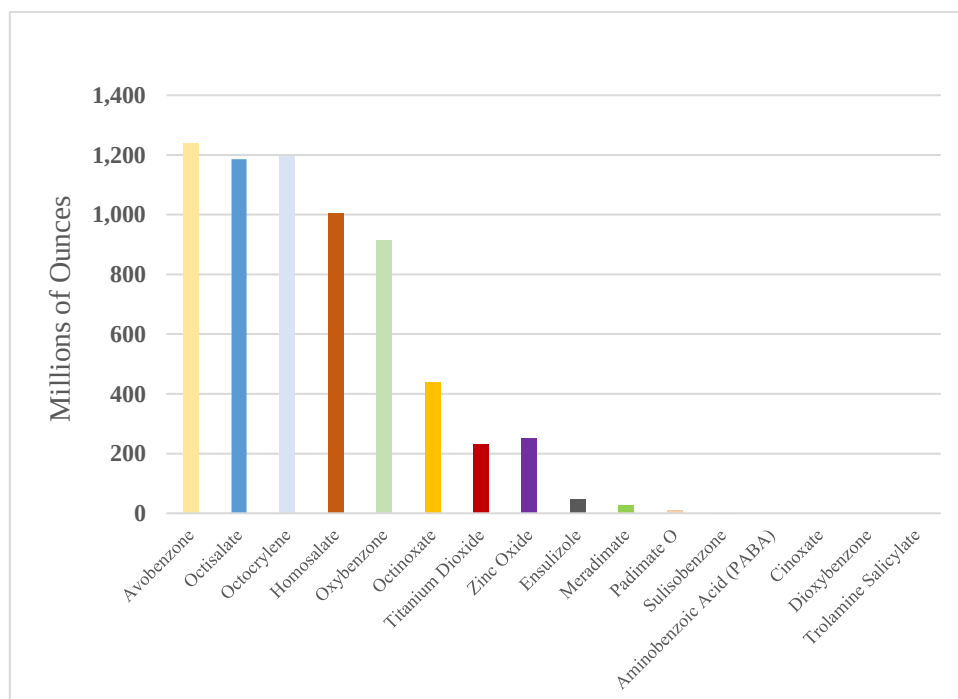


Figure 1.5. FDA-estimated upper bounds of 2016 U.S. sunscreen active-ingredients (millions of ounces) (U.S. Food & Drug administration, 2019).

Organic UV filters are regarded as pseudo-persistent environmental contaminants; however, currently, there is limited knowledge regarding their distribution and effects on

aquatic systems (Bachelot et al., 2012). Although toxicity research on UV filters has focused largely on freshwater taxa, several studies have evaluated marine species across trophic levels-spanning primary producers, fish, and reef-building corals. In marine ecosystems, the effects of UV filters may be direct or mediated through bioaccumulation and transformation products, with documented consequences including acute and chronic toxicity, genotoxicity, cellular stress and antioxidant responses, reproductive impairment, developmental and behavioral alterations, coral bleaching, and endocrine disruption (Carve et al., 2021; Lozano, Givens, et al., 2020). Mounting evidence raises concerns about their safety: UV filters have shown toxicity toward standard test organisms such as algae (Mao et al., 2017; Sieratowicz et al., 2011; Thorel et al., 2020), corals (Conway et al., 2021; Corinaldesi et al., 2018; Danovaro et al., 2008), crustaceans (Park et al., 2017; Thorel et al., 2020), fish (Douglas, 2003; Downs et al., 2016; Kunz & Fent, 2006; Sharifan, 2020), and marine bacteria (Lozano, Matallana-Surget, et al., 2020). Moreover, several organic UV filters exhibit endocrine-active properties, with published work linking exposure to reduced fertility and reproductive success (Fent et al., 2008; Kim et al., 2014; Schlumpf et al., 2008; Weisbrod et al., 2007), and to estrogenic activity in vertebrates (Coronado et al., 2008; Kunz & Fent, 2006, 2009; Kunz et al., 2006).

Coral reefs consist of delicate organisms that are highly vulnerable to minor alterations in their environment. Recent studies indicate that cosmetic products, such as sunscreens, play a substantial role in the pollution challenges confronting coral reefs (Burke et al., 2011; Raffa et al., 2019; Wood, 2018). (Burke et al., 2011; Raffa et al., 2019; Wood, 2018). Certain compounds, such as 4-MBC, BP-3, and 2-EHMC, have been linked to negative impacts on reproduction, heightened mortality rates in benthic organisms, coral bleaching, and deformities or death in the early stages of fish development. Several studies indicate that benzophenone-3, 4-methylbenzylidene camphor, and ethylhexyl methoxycinnamate produce negative effects across three marine trophic levels, exhibiting toxicities similar to those of specific heavy metals (Cd, Cu, Hg, Pb, Zn) (Cadena-Aizaga et al., 2022; Cadena-Aizaga et al., 2020).

Exposure to UV radiation can generate reactive oxygen species (ROS), notably superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). Degradation proceeds primarily via photolysis, but can be modified by chlorination processes-particularly in swimming pools-altering transformation pathways. Importantly, the resultant by-product of UV-

filter degradation may present greater hazards than the parent compounds (Santos et al., 2012)

Organic and inorganic filters have been identified in marine environments by water sampling from many locations worldwide. The concentration of contaminants fluctuates based on several circumstances, including location, day and time of measurement, and the presence of swimmers (Astel et al., 2020; Bargar et al., 2015; Tashiro & Kameda, 2013; Tovar-Sánchez et al., 2013). It has been observed that the highest levels of UV filters are found in waters near popular beach destinations, including Hawaii, Okinawa Island, Majorca Island, the Virgin Islands, the Southern Baltic Sea, and the Mediterranean Sea (Tovar-Sánchez et al., 2013). Given these risks, environmental monitoring of UV filters has intensified in recent years (Manová et al., 2015; Park et al., 2017; Ramos et al., 2015; Sharifan et al., 2016). In seawater, BP-3, EHMC, and OC have been reported at 3-118, 5-53, and ~ 32 ng L⁻¹, respectively (Magi et al., 2012; Nguyen et al., 2011). Freshwater concentrations are often higher: treated waters in Spain contained up to 170 ng L⁻¹ OC and 260 ng L⁻¹ EHMC (Díaz-Cruz et al., 2012); in Colombia, river water held 112 ng L⁻¹ OC and 10 ng L⁻¹ EHMC, with sediments reaching 47.1 ng g⁻¹ (dry weight) for EHMC (Barone et al., 2019). In an Australian freshwater recreational reservoir, OC dominated at 1,380-4,660 ng L⁻¹, followed by AVO (4-tert-butyl-4'-methoxydibenzoylmethane) at 424-1,130 ng L⁻¹ (O'Malley et al., 2021). Additional reports list AVO in rivers at 36-38 ng L⁻¹ (Tsui et al., 2014), 2,431 ng L⁻¹ (Rodil et al., 2009), and 20 ng L⁻¹ (Balmer et al., 2005).

1.5. Regulatory Aspects of UV filters

In the European Union (EU), sunscreen and any products formulated with UV filters fall under the scope of the EU Cosmetics Regulations (European Union, 2009). That framework defines UV filters as “*substances which are exclusively or mainly intended to protect the skin against certain UV radiation by absorbing, reflecting or scattering UV radiation*”. Notably, unlike earlier guidance, it does not address protection of the cosmetic product itself from light (Chisvert & Salvador, 2007), such wording appeared in the preceding EU Cosmetics Directive (Directive 76/768/EEC) (European Union, 2003).

Annex VI of Regulations (EC) No. 1223/2009 lists the UV filters that are permitted for use in cosmetic products, each with specific maximum concentrations and use conditions.

The accompanying Table 2 summarizes the currently authorized UV filters in Annex VI, reflecting the most recent amendments. Safety evaluations for individual filters are issued by the Scientific Committee on Consumer Safety (SCCS), which provides opinions based on available evidence; these assessments focus on consumer safety and may reference broader considerations where relevant (Buzek, 2009). Under the EU framework, only UV filters included in Annex VI may be used in cosmetics; substances not listed are prohibited. Notably, benzyl salicylate-used as both a fragrance and a potential UV-absorbing ingredient-does not appear in Annex VI but is restricted under Annex III due to specific labelling requirements. The regulatory status is frequently updated and has generally become more stringent where health concerns arise. For example, PABA and 3-benzylidene camphor, initially allowed in 2009, were subsequently banned in 2013 and 2015, respectively; zinc oxide was later added as an allowed substance; and the maximum concentration of benzophenone-3 was reduced from 10% to 6% in 2017. At the time of the cited amendment, 25 organic and 2 inorganic UV filters were authorized; Table 1.2 provides the full list with limit values.

Table 1.2: Specific restrictions of the studied UV filters according to regulation 1223/2009 (Buzek, 2009).	
EHS	5%
HMS	10%
BP -3	6% and it must be indicated in the label ‘Contains benzophenone-3’ if the concentration exceeds 0.5 % (not for product protection purposes).
BP -4	5% (as acid)
IAMC	10%
EHMC	10%
4MBC	4%
OCR	10% (as acid)
EHPABA	8%

The EU framework also considers environmental dimensions of UV-filter use, recognizing some sunscreen ingredients as emerging aquatic contaminants. In July 2022, AVO, OCR, and BP-3 were added to the EU surface-water watch list owing to potential environmental risks (European Union, 2022).

In the United States, sunscreens are regulated by the Food and Drug Administration (FDA) as over-the-counter (OTC) drugs-unlike the European Union, where they are treated as cosmetics so they must undergo FDA review and comply with stringent labeling requirements (DeLeo, 2019). This framework has constrained the number of UV-filter actives available in U.S. products relative to jurisdictions that authorize additional ingredients (Pantelic et al., 2023; Wang & Lim, 2011). In its 2019 proposal, the FDA categorized sunscreen actives as: GRASE (Category I), non-GRASE (Category II), or requiring additional data (Category III). Titanium dioxide and zinc oxide were designated GRASE (Category I), whereas other organic filters were placed either in Category II or, more commonly, in Category III pending further evidence (Federal Register 84FR6204-6275, 2019-03019) (Records, 1977; Sabzevari et al., 2021). The FDA's 2021 proposed final administrative order further codified standardized data requirements for the organic filters that remained "not yet GRASE" (Category III), reflecting an ongoing, evidence-based safety review (H. W. Lim et al., 2022).

Availability and permitted concentrations of organic UV filters (UVFs) differ by jurisdiction. For instance, U.S. regulations allow up to 6% BP-3 and 7.5% EHMC in cosmetic formulations, whereas the European Union permits higher inclusion levels-reported as up to 10% for both BP-3 and EHMC (Schaap & Slijkerman, 2018). Sunscreen labeling schemes based on SPF also vary internationally: Australia classifies SPF 4-14 as low protection, 15-29 medium, 30-59 high, and ≥ 60 very high; the EU designates SPF 6-14 as low, 15-29 medium, 30-49 high, and ≥ 50 ultra-high. By contrast, the US. FDA does not tier SPF into categories, emphasizing use of at least SPF 15 (often SPF 30-50 for fair-skinned users). For UVA protection, Japan's JCIA employs the PA system (PA^+ to PA^{++++}), derived from the *in vivo* persistent pigment darkening method (Breakell et al., 2024).

1.6. Analytical techniques for UV filters

Advanced chromatographic and mass spectrometric methods, such as high performance liquid chromatography (HPLC) with diode-array detection (Lu et al., 2021; Mei & Huang, 2017), HPLC-UV (Suárez et al., 2016; Wang et al., 2019), liquid chromatography–mass spectrometry (LC-MS) (Cheng et al., 2018; Zenker et al., 2008), or tandem mass spectrometry (LC-MS/MS) (Carve et al., 2023; Mokh et al., 2022; Pafili et al., 2021), and gas chromatography–mass spectrometry (GC-MS) (Mokh et al., 2022; Trujillo-Rodríguez et al., 2020), or GC-MS/MS (Yücel et al., 2022), are now routinely employed to identify and quantify organic UV filters in environmental waters because they offer high sensitivity with robust separation performance. A literature survey of peer-reviewed studies published since 2006 reported that trace-level determinations of organic UV filters in environmental matrices are achieved comparably by GC- or LC-based workflow when coupled to MS or tandem MS detection (Oubahmane et al., 2023). The surge in LC-MS/MS applications over the past two decades reflects considerable advances in both instrumentation and method development. For relatively clean aqueous matrices (e.g., bathing waters), costly high-end MS detectors may be unnecessary; common optical detectors, such as UV-photodiode array (PDA), often provide adequate performance for UV-filter quantification (Cadena-Aizaga et al., 2022).

GC is well-suited for identifying and quantifying thermostable volatile or volatilizable analytes. For structurally diverse organic UV filters present at trace levels in complex environmental matrices, GC can be advantageous, particularly when multi-residue methods are used to measure UV filters alongside other priority or emerging contaminants with high efficiency and accuracy (Biel-Maeso et al., 2019; Celeiro et al., 2019; Pintado-Herrera et al., 2013). GC-MS analyses commonly employ derivatization, which enhances thermal stability and volatility, sharpens chromatographic peaks (reducing tailing) for hydroxylated UV filters, and often lowers method detection limits while improving sensitivity relative to the native compounds. Derivatives can also yield more diagnostic fragmentation and characteristic ions for MS detection under selected-ion monitoring (SIM) and related modes. The principal drawback is that derivatization adds time and procedural complexity (Cadena-Aizaga et al., 2020; Gago-Ferrero et al., 2013). Finally, matrix-induced signal suppression/enhancement is typically less problematic in the ionization regimes used for GC-MS than in many LC-MS workflows, which contributes to the robustness of GC for real-sample analysis.

For mixtures of organic UV filters spanning a wide range of physicochemical properties, liquid chromatography coupled to tandem mass spectroscopy (LC-MS/MS) is generally the method of choice (Benedé et al., 2014; Tsui et al., 2014). It is also particularly suited to concurrent quantification of parent compounds and their typically more polar transformation products (TPs) (Gago-Ferrero et al., 2013).

Matrix effects can compromise quantitative determinations of organic UV filters in environmental samples for both GC- and LC-based methods because co-extracted components alter analyte ionization during MS detection. In GC-MS, these effects are typically less pronounced, especially for thermally stable UV filters and when co-eluted lipids are minimal, whereas LC-MS often experiences stronger suppression or enhancement. Despite this, LC remains the most widely applied platform for UV-filter analysis. Consequently, highly sensitive and selective approaches are needed to mitigate matrix effects; where they cannot be eliminated, they must be corrected to avoid bias in measured concentrations (Cadena-Aizaga et al., 2020).

In addition to liquid and gas chromatography, a variety of alternative techniques exist. Micellar electrokinetic chromatography (MEKC) employs uncoated fused-silica capillaries alongside sodium dodecyl sulfate micelles, which function as a pseudo-stationary phase. Alternative methods comprise thin-layer chromatography, supercritical fluid chromatography, and square-wave voltammetry (Narloch & Wejnerowska, 2021).

2. The UV filter 2-Ethylhexyl methoxycinnamate (EHMC)

This thesis specifically examines the ultraviolet (UV) filter EHMC, which is among the most prevalent UV filters used in personal care items.

2.1. Introduction

EHMC, also referred to as octinoxate or Uvinul MC80, is among the most widely used organic UV filters in sunscreen formulations. Commercial EHMC is an isomeric mixture dominated by the *trans* form (>80%; 2-ethylhexyl *trans*-4-methoxycinnamate, EC: 629-661-9, CAS: 83834-59-7) has been registered under REACH as 2-ethylhexyl 4-methoxycinnamate prior to 2013 (EC: 226-775-7, CAS: 5466-77-3 (Buzek, 2009; Lorigo et al., 2018)). The molecule bears a *para*-methoxy electron-donating substituent and an electron-withdrawing 2-ethylhexyl propenoate ester in the aromatic ring, creating an extended conjugated system that enables efficient absorption near 310 nm within the UVB range (Miranda et al., 2014), with a high molar absorptivity ($\epsilon \sim 19,500 \text{ M}^{-1} \text{ cm}^{-1}$). Upon solar irradiation, it photoisomerizes to the less stable *cis* form, whose λ_{max} is near 312 nm, but with markedly lower absorptivity ($\epsilon \sim 10,000 \text{ M}^{-1} \text{ cm}^{-1}$), thereby diminishing overall sunscreen efficacy (Nečasová et al., 2017; Shaath, 2010; Sharma et al., 2017).

EHMC is widely incorporated into personal-care formulations, including cosmetics and sunscreens, to provide photoprotection for skin. Beyond its role as a sunscreen active, EHMC functions as a UV stabilizer to limit photodegradation in numerous personal care, laundry, and cleaning products (He et al., 2024; Nagorka & Duffek, 2021). It is classified among the EU High Production Volume Chemicals (HPVC), indicating production or import exceeding 1000 t yr⁻¹ within the EU (Vione et al., 2015). Extensive use, combined with skin wash-off during activities such as swimming, bathing, and incomplete removal in wastewater treatment, has resulted in broad environmental and human exposure (Huang et al., 2019). Reported concentrations include up to 0.075 µg L⁻¹ in river water (Da Silva et al., 2015), 1,410 ng L⁻¹ in seawater (Fenni et al., 2022), and 9.6 µg L⁻¹ in wastewater influent (Balmer et al., 2005).

2.2. Environmental and toxicological implications

Emerging evidence indicates that EHMC poses substantial hazards to aquatic and vertebrate development. In zebrafish, acute exposures of 12.4-62 mg L⁻¹ from 3 to 96 hours post-fertilization (HPF) produce developmental defects, including pericardial edema, spinal curvature, and tail malformations (Nataraj et al., 2020). In mammals,

maternal exposure in mice to 1000 mg kg⁻¹ during lactation impaired offspring development, evidenced by delayed eye opening, reduced weight gain in both sexes, and decreased circulating T4 in pups (Ferraris et al., 2020). Mechanistically, EHMC elicits developmental neurotoxicity in zebrafish, marked by excessive apoptosis in cranial and spinal regions, elevated reactive species and malondialdehyde, and depleted glutathione (Yang et al., 2024). Further research indicates that EHMC reduces thyroid hormone levels and disrupts the expression of genes associated with thyroid function, neurotoxicity, and nephrotoxicity, aligning with hypothyroidism and wider toxicological reactions in both adult and larval zebrafish (Chu et al., 2021). At environmentally relevant concentrations, EHMC also alters hepatic antioxidant gene transcripts in zebrafish (Zhou et al., 2019).

Toxic effects of EHMC have also been documented in adult nubbins of the reef coral *Seriatopora caliendrum*. In single-compound assays, mortality (33.3%) and extensive bleaching (83.3%) occurred only at the highest EHMC dose tested (1000 mg L⁻¹). In mixture exposures, co-treatment with octocrylene produced high mortality in both species examined and triggered bleaching of *Seriatopora caliendrum* even at 1 mg L⁻¹, indicating enhanced toxicity under combined UV-filter exposure (He et al., 2019). In *Daphnia magna*, a 48-h exposure to 4 mg L⁻¹ caused ~50% mortality, whereas 1 mg L⁻¹ was indistinguishable from controls (Jang et al., 2016). In another study, in *Daphnia magna*, exposure increased catalase activity, indicating activation of antioxidant defenses (de Paula et al., 2022).

Development of the brackish polychaete *Ficopomatus enigmaticus* was disrupted at the larval stage, and adults showed neurotoxic responses under EHMC exposure (Cuccaro et al., 2022; Sanches et al., 2021). Evidence of maternal transfer has also been reported in small cetaceans: in paired mother-fetus samples, franciscana dolphins exhibited EHMC levels of 43-54 ng g⁻¹ (lipid weight, l.w.) in maternal muscle, versus 70-250 ng g⁻¹ l.w. in fetuses; the compound was additionally detected in milk (~120 ng g⁻¹ l.w.) and placenta, implying efficient transplacental and lactational transfer (Alonso et al., 2015). *In silico* assessments indicate that degradation products formed after EHMC isomerization may be genotoxic and induce chromosomal aberrations [128], and chlorination byproducts of EHMC have demonstrated mutagenic potential *in vitro* (Nakajima et al., 2009).

2.3. Photodegradation of EHMC

After absorbing UV photons, organic UV filters such as EHMC are promoted to excited states that relax back to the ground-state via photophysical pathways (radiative or non-radiative) and/or photochemical reactions. Unlike purely photophysical deactivation, photochemical routes generate new products that may be photoinactive, reducing overall sunscreen protection, or may interact with skin biomolecules, trigger photosensitivity, or react with co-formulated UV filters/ingredients (Duarte et al., 2019). EHMC is particularly sunlight-labile: the thermodynamically favored *trans* isomer present in the dark undergoes rapid *trans/cis* Photoisomerization under UV irradiation. The photostationary *trans/cis* ratio is strongly influenced by the surrounding medium, especially its polarity (Herzog et al., 2019; Nečasová et al., 2017). Comparative summary of EHMC photodegradation studies are described in the Table 2.1.

2.4. Type of Photodegradation pathways

2.4.1. Direct photolysis

EHMC undergoes direct photolysis: UV absorption by its cinnamate chromophore promotes excited states that drive bond cleavage and structural rearrangements. This pathway is most pronounced under UVB, where EHMC's absorption is strongest. The commercially predominant *trans* isomer therefore undergoes photochemical transformation, yielding multiple identifiable products (Jentzsch et al., 2016; MacManus-Spencer et al., 2011; Vione et al., 2015). Vione et al. (Vione et al., 2015) conducted a study on the environmental photodegradation of EHMC in ultrapure water under simulated sunlight and found that the primary pathways for EHMC degradation is direct photolysis. This process leads to the formation of various transformation products, such as 4-methoxybenzaldehyde (4-MBA), some of which are more toxic than the parent compound. The study suggest that the transformation products of EHMC pose significant environmental risks.

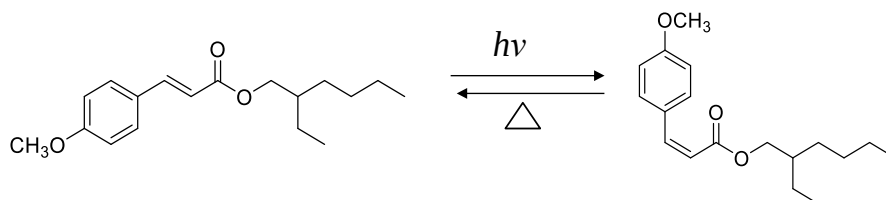
2.4.2. Indirect photolysis

Indirect photolysis occurs via sensitization: photochemically generated oxidants, such as hydroxyl radicals ($\cdot\text{OH}$), singlet oxygen, and other reactive oxygen species (ROS) arising from dissolved organic matter or co-present photosensitizers, attack EHMC. These species promote oxidative pathways, including hydroxylation and related transformations

of the parent molecule (Lu et al., 2023; MacManus-Spencer et al., 2011; Vione et al., 2015).

2.4.3. Photoisomerization

Photoisomerization is a principal photochemical pathway for EHMC, converting the *trans* isomer to the *cis* form (Scheme 2.1). Because the *cis* isomer exhibits a shifted, weaker UV absorption profile, this interconversion diminishes overall UV protection. Under UV irradiation, the reaction proceeds rapidly and approaches a photostationary state in which *trans* and *cis* coexist at characteristic ratios. In solution, the predominant *trans*-to-*cis* conversion is well documented, but reported losses in EHMC absorptivity span a wide range: some studies (Huong et al., 2007; Morliere et al., 1982; Pattanaargson & Limphong, 2001; Pattanaargson et al., 2004) describe near-irreversible degradation with up to ~90% loss, whereas others observe a *trans/cis* photostationary mixture with only ~30-50% reduction. In commercial formulations, where EHMC (also called OMC) is combined with other organics, absorbance decreases reported vary from ~10% to ≥90%, reflecting differences in matrix, photon flux, and methodology. Variability arises from disparate experimental settings, including sample form (dilute solution, film, and creams), lamp type, irradiance, and solvent system (Hanson et al., 2015; Huong et al., 2007; Pattanaargson et al., 2004).



Scheme 2.1. Isomerization of EHMC (*trans/cis*).

The photodegradation of EHMC has been studied extensively under various conditions, highlighting its photoisomerization and the formation of potentially toxic degradation products. Duarte et al. (Duarte et al., 2019), explored the photodegradation of EHMC under simulated solar light, noting that exposure led to a significant reduction in the *Trans* form, which degraded by up to 86% after just 1 hour of irradiation. However, encapsulated in alginate microplastics, particularly with Vitamin E, the degradation was significantly reduced, with only 68% and 57% degradation in EHMC-MP and EHMC+ Vitamin E-MP, respectively. This study underscores the role of encapsulation in enhancing the photostability of EHMC by limiting photoisomerization and suggest that

antioxidants such as Vitamin E can further suppress the formation of the *cis* isomer. Similarly, Gackowska et al. (Gackowska et al., 2014), studied the photoisomerization of EHMC in the presence of reactive oxygen and chlorine species and confirmed the formation of both the *trans* and *cis* isomers. They also observed that UV exposure in the presence of hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) accelerated the photodegradation process, further emphasizing the role of environmental factors in the isomerization dynamics.

As EHMC undergoes photoisomerization, it also generates various degradation products. Some of these products, including 4-MBA and 4-methoxycinnamic acid (4-MCA), have been identified as toxic. The study by Gackowska et al. (Gackowska et al., 2018), explored the toxicity of EHMC degradation products and found that chlorinated derivatives, such as chlorophenols, exhibited significantly higher toxicity than EHMC itself. These byproducts were found to be particularly harmful to aquatic organisms, like *Daphnia magna*, and bacteria, suggesting a significant environmental risk associated with EHMC photodegradation. In another study, several photo-transformation products (photo-TPs) of EHMC through HPLC-UV and LC-ESI-MS analyses. They found that the degradation rate follows first order kinetics with some of these transformation products exhibiting genotoxic effects. This highlights the environmental implication of EHMC's photodegradation, especially concerning greywater reuse and potential for long term ecological impact (Jentzsch et al., 2016).

The photodegradation rate of EHMC is also influenced by the medium in which it is exposed to UV light. In oil-in-water emulsion, such as those used in sunscreens, Herzog et al. showed that EHMC undergoes a second-order [2+2] photocycloaddition reaction, which forms dimers that are inactive as UV absorbers. Interestingly, they found that the degradation rate decreases with higher EHMC concentrations due to a higher optical density, which limits photon absorption. This finding emphasizes the importance of oil polarity and EHMC concentration in sunscreen formulations for maintaining the filter's photostability (Herzog et al., 2019).

Table 2.1: Summary of the photodegradation studies of EPMC under different condition.					
Matrix	Light source	Isomerization	Time of Exposure	Result	Reference
Aqueous solution	UV light (medium pressure mercury lamp, 200-400 nm)	EPMC photodegradation occurred alongside OCR, forming mixed TPs.	0-256 minutes.	EPMC degraded faster than OCR, with mixture effects observed in the TPs formation and degradation patterns	(Jentsch et al., 2019)
Aqueous solution	Simulated sunlight and natural sunlight.	Photoisomerization of OMC (EPMC) E/Z isomerization observed.	30 minutes-several hours	Photolysis resulted in E/Z isomerization and dimer formation (cyclobutane dimers). Direct and indirect photolysis occurred	(MacManus -Spencer et al., 2011)
Aqueous and organic solution	Steady-state and laser flash photolysis	Photoisomerization E/Z with no intermediates detected.	Several minutes	High photoisomerization quantum yields (0.5-1). Photostationary state reached E/Z isomerization	(Morliere et al., 1982)

Continued

Table 2.1: Summary of the photodegradation studies of EPMC under different condition.					
Matrix	Light source	Isomerization	Time of Exposure	Result	Reference
Oil-in-water emulsions (sunscreens)	Solar simulator (UVB+UVA)	Trans to cis isomerization with [2+2] photocycloaddition forming inactive photoproducts.	1-10 hours (UV dose: 29.9 J cm ⁻² per hour)	The reaction follows a second order rate law; less EPMC degradation at higher concentrations, and higher photostability with non-polar oils.	(Herzog et al., 2019)
Solvent (Hexane, Diox, AcOEt, THF, iPro, CAN water) commercial sunscreens	UV irradiation (various solvents)	Rapid E/Z isomerization upon irradiation.	varies	Photoisomerization of OMC (EPMC), especially under sunlight or UVA exposure, reduces SPF effectiveness.	(Huong et al., 2007)
Aqueous solution	UV light (medium pressure mercury lamp, 200-400 nm)	Formation of oxidized and hydroxylized EPMC isomers.	4-256 minutes	EMC photodegradation followed first-order kinetics, with oxidized photo-TPs formed.	(Jentsch et al., 2016)

Continued

Table 2.1: Summary of the photodegradation studies of EPMC under different condition.					
Matrix	Light source	Isomerization	Time of Exposure	Result	Reference
Ultrapure/bidistilled water, spiked with stock in methanol	Artificial sunlight (SOL500 lamp, 290-800 nm, quartz UVA+UVB), vials	Rapid E/Z isomerization within first 7 h; E/Z $\approx$ 2.5 after 7h; photostationary ratio	Up to 72 h; half-life: EPMC $\approx$ 21 h	Photodegradation with polymerization (first order kinetics). Formation of dimers (detected by LC-MS). Both cis and trans isomers decrease after prolonged irradiation parent EPMC eliminated by sunlight	(Rodil et al., 2009)
Aqueous solution (ultrapure water spiked with EPMC)	Artificial lamps: Philips TLK 05 (365 nm UVA) and Philips TL 01 RS (313 nm UVB) lamps; natural sunlight conditions modeled	No photoisomerization observed in water and dimerization major hydroxylation pathways; photoisomerization favored in organic solvents but not in water.	4 h of irradiation (direct photolysis); photocatalysis with TiO ₂ also studies	EPMC undergoes rapid direct photolysis mainly under UVB, with half-life $\sim$ 30 min (UVB) and $\sim$ 250 min (UVA), forming TPs including 4-MBA, hydroxylated and dimeric derivatives. Indirect photolysis via ROS negligible. Photocatalysis accelerates degradation with additional TPs.	(Vione et al., 2015)

Continued

Table 2.1: Summary of the photodegradation studies of EPMC under different condition.					
Matrix	Light source	Isomerization	Time of Exposure	Result	Reference
Methanol solution	Sunlight exposure	E/Z photoisomerization	Varies (exposure over several days)	The Z-isomer of EPMC was isolated after sunlight exposure confirming the E/Z isomerization process	(Pattanaar gson & Limphong , 2001)
Methanol, ethanol, hexane	Sunlight exposure	E/Z photoisomerization with changes in UV absorbance	Varies (sunlight exposure for several hours)	E/Z photoisomerization equilibrium was monitored, showing dependence on solvent polarity and concentration	(Pattanaar gson et al., 2004)

3. Bisphenols

3.1. Introduction

Bisphenol A (BPA) was first synthesized by Alexander Dianin in 1891. Polymers of BPA rapidly came into industrial use with the development of polycarbonate plastics in the 1950s, and today, bisphenols are widely used. It is an important industrial chemical and applied in the production of durable and heat resistant food containers, water pipes, sport safety equipment, protective and corrective eyewear, eyeglass lenses, medical equipment and tubing, thermal papers and many plastic consumer products including toys as well as compact discs and DVDs (**Figure 3.1**) (Acevedo et al., 2025). Estimates in 2015 showed annual production of up to 4.85 million tons. The worldwide market in 2022 was approximately 5600 tons, with projections for 2032 indicating a growth of 3.51% (reaching 8000 tons). The Asia Pacific region currently leads globally in consumption, accounting for 55% of the market, with Europe and North America following behind. In the realm of production, the Asia Pacific region represents 35% (Charkiewicz et al., 2024).

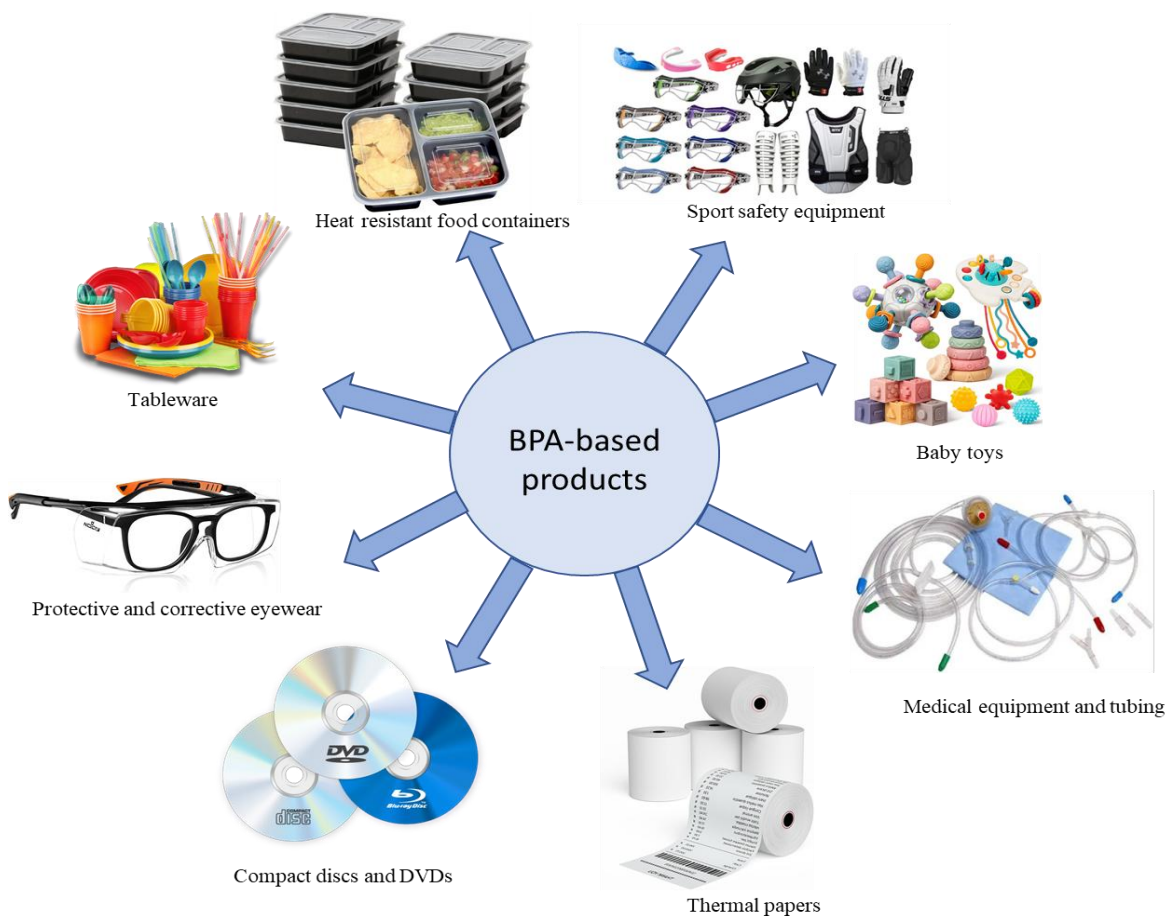


Figure 3.1. Overview of the Bisphenol A sources in industrial and consumer products.

In recent years, endocrine disrupting chemicals (EDCs) have received significant attention due to their widespread presence in environments and potential harm to ecological health. Bisphenol analogues (BPs) are common emerging EDCs and one of the most abundant phenolic compounds, which consist of two phenol groups, mainly including BPA and substituted analogues (Liao et al., 2012; Song et al., 2012). BPA is a carbon-based synthetic compound that belongs to the two-hydroxyphenyl groups of diphenylmethane derivatives and has the chemical formula $C_{15}H_{16}O_2$ and a molecular mass of 228.29 g.mol^{-1} . BPA was once the predominantly used bisphenol. However, extensive research demonstrated its estrogenic activity and possible carcinogenic potential, and it was subsequently classified as an endocrine-disrupting chemical. Industry responded to concerns about BPA by replacing it with other BPs such as BPAF, BPS, and BPF, which will be discussed in the next section. This shift led to the widespread marketing of products labelled “BPA-free”, however, these alternative BPs were introduced without rigorous safety evaluation (Alexander et al., 2025; Lopes-Rocha et al., 2021; Noszczyńska & Piotrowska-Seget, 2018).

3.2. Structural and Physico-Chemical Properties

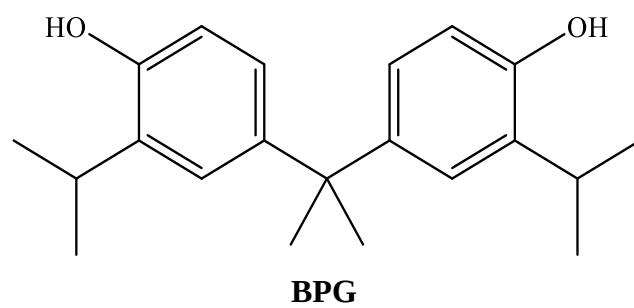
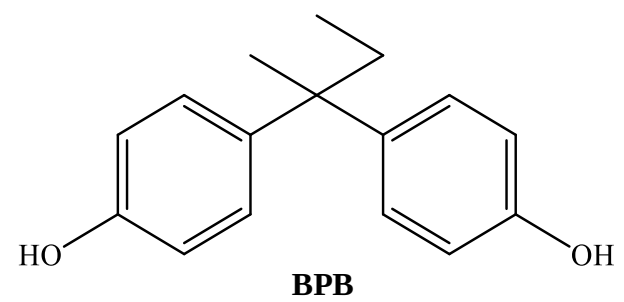
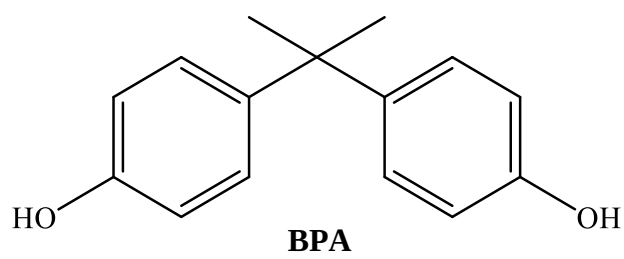
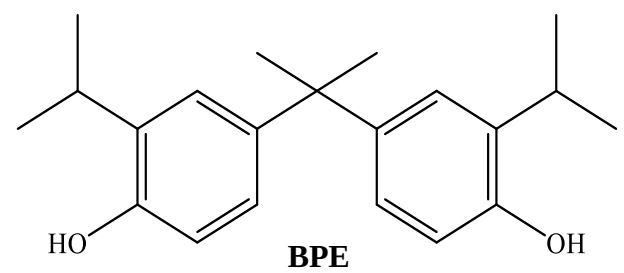
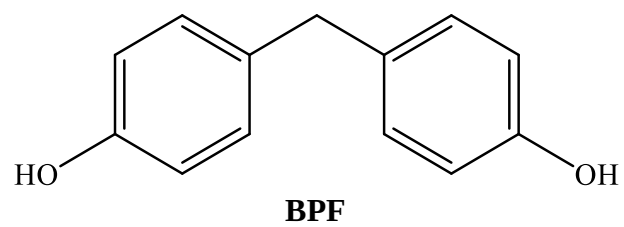
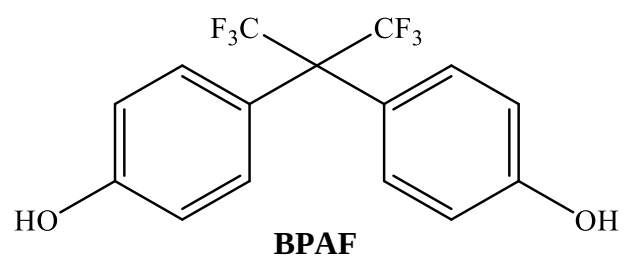
Table 3.1 presents the bisphenols chosen for this study, detailing their identifiers, including INCI (International Nomenclature of Cosmetic Ingredients) name, common abbreviation, molecular formula, molecular weight, CAS number, and various physico-chemical properties. These properties encompass boiling point ($^{\circ}\text{C}$), melting point ($^{\circ}\text{C}$), acidity constant pK_a ($-\log_{10} K_a$), the decimal logarithm of the octanol-water partition coefficient $\log k_{ow}$, (Chen et al., 2016), density, and solubility in water.

A comprehensive collection of data was assembled from ChemSpider (with predicted data generated using the US Environmental Protection Agency’s EPISuite™) as well as from SciFinder and Chemical Book, as detailed in Table 3.1. Figure 3.2 illustrates the relevant chemical structures.

Table 3.1: INCI name, Molecular formula, molecular weight, CAS No., and some physico-chemical properties of studied bisphenol compounds.										
INCI name	Molecular Formula	MW (g mol-1)	CAS No.	Boiling Point (°C)	Melting Point (°C)	Acidity constant pKa	logKow [9]	Density	Half-Life (day) in water [8]	Solubility in water
4,4'-(Hexafluoroisopropylidene)diphenol (BPAF)	C ₁₅ H ₁₀ F ₆ O ₂	336.23	1478-61-1	400	160-163	8.74±0.10	4.47	1.3837	180	Insoluble
4,4'-Methylenediphenol (BPF)	C ₁₃ H ₁₂ O ₂	200.23	620-92-8	390.0±22.0 °C at 760 mmHg	162-164	9.91±0.10	3.06	1.2±0.1 g/cm ³	15	Insoluble
4,4'-Ethylidenebisphenol (BPE)	C ₁₄ H ₁₄ O ₂	214.26	2081-08-05	215	123-127	10.10±0.10	3.19	1.171±0.06 g/cm ³	15	-
4,4'-Isopropylidenediphenol (BPA)	C ₁₅ H ₁₆ O ₂	228.29	80-05-7	220 °C / 4 mm Hg	158-159	10.29±0.10	3.32	1.195	37.5	<0.1 g/100 mL at 21.5 °C

Continued

Table 3.1: INCI name, Molecular formula, molecular weight, CAS No., and some physico-chemical properties of studied bisphenol compounds.										
INCI name	Molecular Formula	MW (g mol-1)	CAS No.	Boiling Point (°C)	Melting Point (°C)	Acidity constant pKa	logKow (Ma et al., 2019)	Density	Half-Life (day) in water [8]	Solubility in water
4,4'-(1-Methylpropylidene)bisphenol (BPP)	C ₁₆ H ₁₈ O ₂	242.31	77-40-7	245.14	126	10.27±0.10	4.13	1.0537	37.5	Insoluble
4,4'-(Propane-2,2-diyl)bis(2-isopropylphenol) (BPG)	C ₂₁ H ₂₈ O ₂	312.4	127-54-8	195 °C / 0.6mmHg	98	10.38±0.10	6.55	1.042±0.06 g/cm ³	37.5	Slightly soluble
1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethylene (BPC2)	C ₁₄ H ₁₀ Cl ₂ O ₂	281.1	14868-03-2	405.0±40.0 °C	213-217	9.86±0.15	3.75	1.407±0.06 g/cm ³	37.5	
4,4'-(cyclohexane-1,1-diyl)diphenol (BPZ)	C ₁₈ H ₂₀ O ₂	268.3	843-55-0	440.9±38.0 °C at 760 mmHg	190-192 °C	9.91±0.30	5.00	1.2±0.1 g/cm ³	37.5	Insoluble



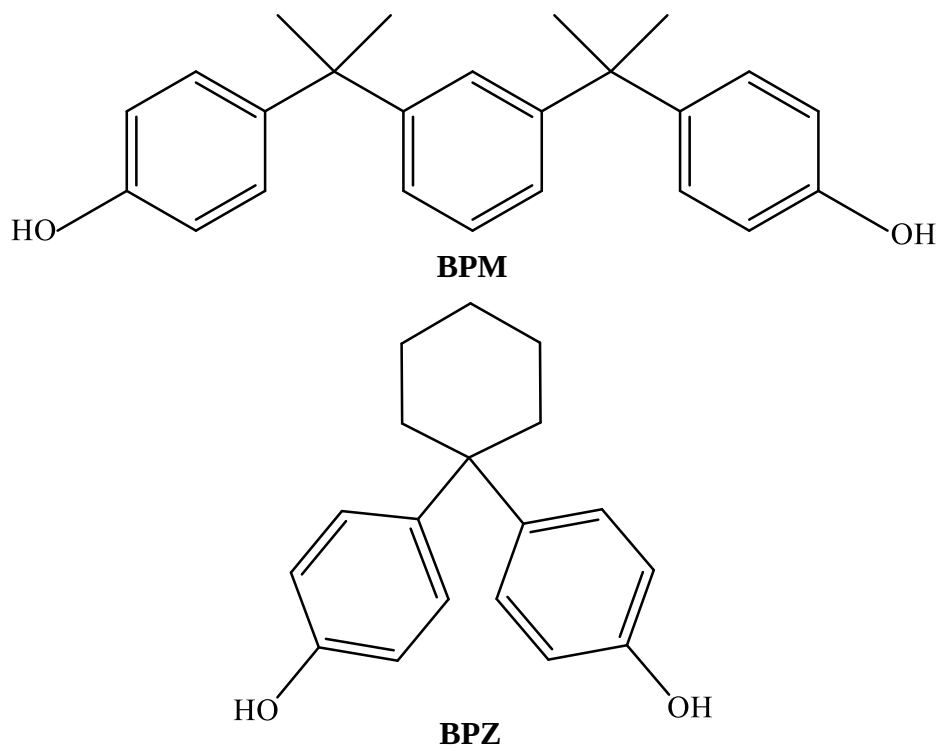


Figure 3.2. Chemical structures of the studied BP compounds.

3.3. Regulatory aspects of bisphenols

Legislative perspectives on the safety implications of BPA in package production remain significantly divergent. The EU maintains a highly precautionary approach, regularly revising legalization to reflect scientific consensus and stakeholder input. EFSA's 2023 opinion concluded that dietary exposure to BPA poses health concerns for consumers of all ages. After examining over 800 scientific studies from 2013 onward, EFSA experts found evidence for immune system impacts, including increased T-helper cells implicated in allergy and autoimmune disorders. Other systems include reproductive, developmental, and metabolic were also flagged for potential harm. In response, EFSA lowered the tolerable daily intake (TDI) for BPA dramatically to 0.2 ng/kg body weight per day, a 200,000 fold reduction from previous limits. This dramatic shift underscores the regulatory urgency and justified the resulting bans enacted by the European commission (European Food Safety Authority, 2023). In 2024, the European commission officially adopted a sweeping ban on BPA and derivatives, taking into account EFSA's risk assessment in all materials intended for food contact including coatings on cans, bottles, and kitchenware following EFSA's scientific evaluation that flagged harmful effects on the immune system. The process featured public consultation, extensive EU member state participation, and allowed an 18 month phase-out for most industries to adapt; with limited exceptions where no safe alternatives exist (European Food Safety Authority, 2025; European Commission, 2024).

The nations of Denmark, Sweden, and Belgium have implemented limitations on the use of BPA-containing substances in food packaging intended for children aged 0 to 3 years. In contrast, Sweden has specifically limited these restrictions to products used in paints and protective coatings for food packaging for the same age group. Since October 2010, Austria has implemented restrictions on the use of this xenoestrogen in pacifiers and containers (Dias et al., 2022; Fonseca et al., 2022).

In the United States, the FDA has amended its food additive regulations to prohibit the use of BPA-based materials but only in baby bottles, spill proof cups, and infant formula packaging. These bans, however, are clearly stated by the FDA as "not based on safety". Instead, the FDA approved these bans at the request of the American Chemistry Council since consumer avoidance of BPA containers forced manufacturers to abandon BPA containing packaging and the FDA pronouncement had the benefit of clearing up market confusion as to whether BPA is present (U.S. Food & Drug administration, 2014). FDA

banned BPA in baby bottles and sippy cups in 2012, in response to industry changes but maintains that BPA is safe at current exposure levels in most of the food contact materials. The EPA lists BPA as a “chemical not concern”, but comprehensive consumer bans have not matched the EU’s (EFSA Panel on Food Contact Materials et al., 2023).

Canada was the first country to ban BPA in baby bottles in 2010. Subsequent regulations set migration and use limits, with ongoing risk reviews by Health Canada (Crozier-Haynes, 2010; Kiss, 2014). In alignment with their precedent, these prohibitions have been implemented in the United States, several European nations, and various Asian countries (Ma et al., 2019). In the meantime, the Australian and New Zealand Food Safety Authority overlooked the potential health risks associated with BPA consumption, particularly concerning infants using baby bottles that contain this xenoestrogen. A study conducted in Australia in 2016 confirmed that dietary exposures among consumers were low and remained within acceptable, safe limits. In New Zealand, this matter remains unregulated, and numerous companies continue to utilize it in the production of baby products (Downs et al., 2016).

3.4.Sources and environmental fate

BPA and its derivatives such as BPS, BPF, BPE, BPB, BPZ and BPAF might be released by the imperfect polymerization of the plastic substances that appears during processing or depolymerisation due to deliberate heating for sterilization or accidental purposes during storage (Figure 3.3) (Alexander et al., 2025; Lopes-Rocha et al., 2021; Noszczyńska & Piotrowska-Seget, 2018). BPA may be emitted into the environment at various points throughout a product's life cycle, including during its production, use, or disposal (European Environment Agency, 2023; Ma et al., 2019). They can further be transported over long distances through atmospheric deposition, rainfall runoff, and other pathways (Liu et al., 2020; J.-L. Zhao et al., 2021).

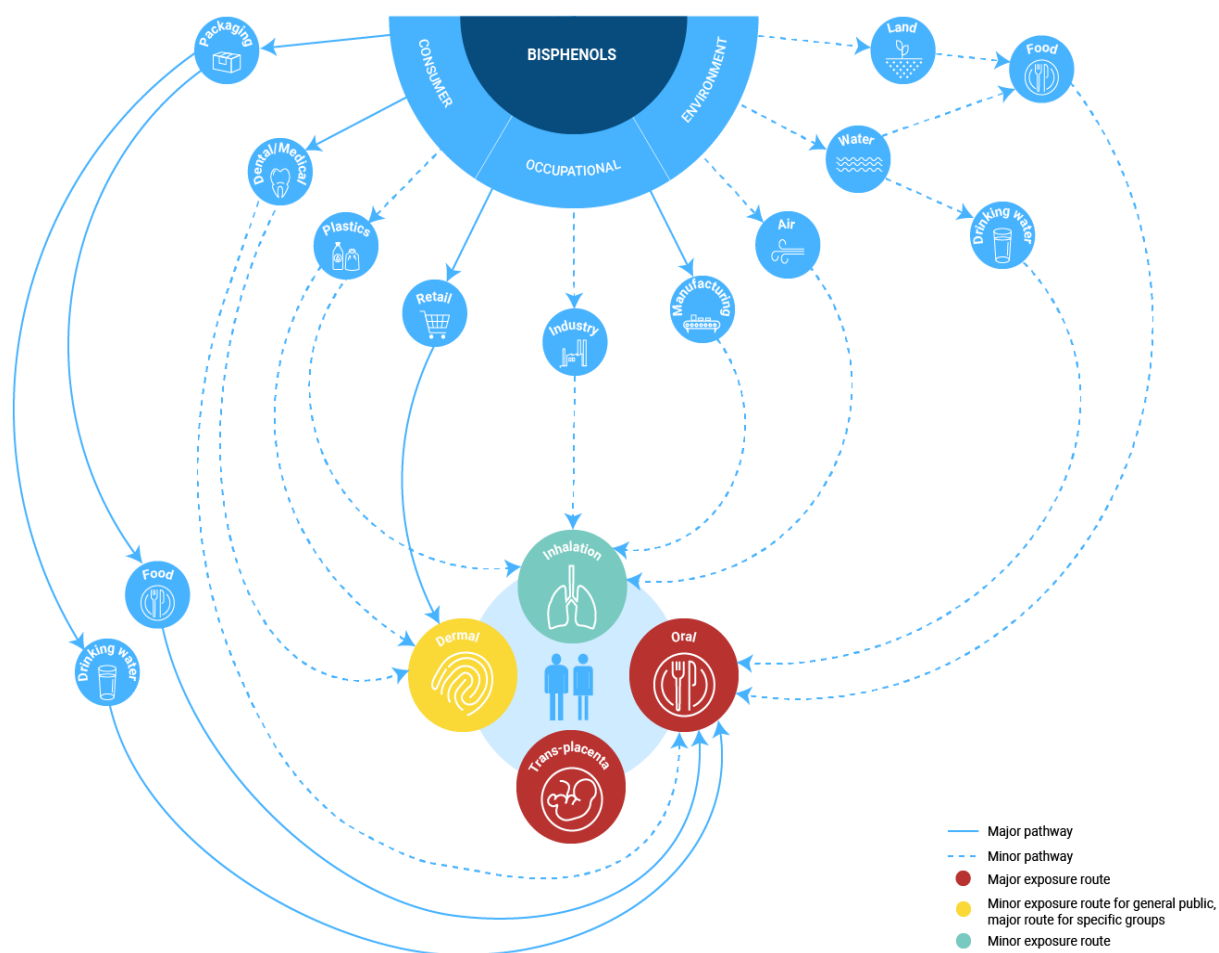


Figure 3.3. Pathways of Bisphenol exposure and potential routes of human contact (European Environment Agency, 2023).

Environmental behavior (e.g., transport and bioavailability) and fate of organic chemicals can be greatly influenced by their physicochemical properties. Data on the physicochemical properties of BPs is limited in the Table 3.1, but still could be used to explain their environmental fate. Bisphenols with octanol-water partition coefficient $\log K_{ow} \leq 4$, such as BPB, BPE, BPF, BPA, and BPC2 are expected mostly in the water phase. Other BPs such as BPAF, BPG and BPZ have $\log K_{ow}$ of 4–6 and have the potential to adsorb to soil and sediment and to accumulate into the fat tissues of living organisms (Caballero-Casero et al., 2016; Chen et al., 2016). BPs in rivers often bind to particulate matter and settle to the bottom, where sediment can become an important sink and storage reservoir for BPs (Hu et al., 2019). However, similar to BPA, which is subject to rapid metabolism (Dekant & Völkel, 2008), other analogues may be metabolized at similar rates, thus reducing their bioaccumulation potencies. The biodegradability of bisphenol analogues in seawater was ranked as BPF $\gg$ BPA $>$ BPP $>$ BPE $>$ BPB $\gg$ BPS. BPF degrades at a

faster rate than BPA, whereas BPS, BPB and BPP are more resistant to biodegradation than BPA in aquatic environments (Danzl et al., 2009; Ike et al., 2006; Sakai et al., 2007).

At the interface of production, use, and disposal, wastewater treatment plants (WWTPs) are major conduits for BPs from both industrial and domestic sources. A multi-site study covering 10 BP analogues reported influent concentrations from ~ 0.90 to 1170 ng L^{-1} , with fate governed by a combination of biodegradation and sorption. In controlled activated sludge bioreactors, BPA, BPF, BPS, BPB, BPE, and BPM showed $> 94\%$ removal, whereas BPAF, BPZ, BPAP, and TCBPA were recalcitrant ($\approx 47\text{-}71\%$ removal) a structure persistence pattern that helps explain why certain “BPA-free” replacements continue to appear in effluents and sludge even as BPA loads decline. Field surveys similarly find non-zero effluent levels and appreciable partitioning to sludge, implying downstream inputs to receiving waters and soils where biosolids are applied (Qian et al., 2021).

Across large river networks, BPA and several analogues (especially BPF and BPS) are now routine detections at low ng L^{-1} levels, with mixture profiles shifting towards BPF/BPS where BPA restriction have tightened (Liu et al., 2021). In the lower Yangtze, a three-year survey (September 2022, 2023, 2024) spanning $\sim 850 \text{ km}$ quantified nine BPs by SPE-LC-MS/MS and reported mean ΣBPs of 67.2 to 28.0 to 46.8 ng L^{-1} (decline-rise pattern), with BPA, BPS, BPF consistently dominant (Liu et al., 2021). Extreme analogues-specific plumes can occur: in the Liuxi River (Guangzhou), BPS ranged across most samples from $0.34\text{-}3,720 \text{ ng L}^{-1}$ with 5 highest values of $19.9\text{-}65,600 \text{ ng L}^{-1}$, far above typical BPA levels in the same catchment (Zhang et al., 2025).

When phase partitioning is resolved, a substantial fraction of riverine BPA resides in the truly dissolved phase, typically on the order of $\sim 1\text{-}6 \text{ ng L}^{-1}$ in base-flowing streams, with a smaller, variable share in the colloidal fraction (Shehab et al., 2020). A targeted seven analogue study likewise found measurable contributions of BPs in both dissolved ($< 5 \text{ kDa}$) and colloidal phases ($5 \text{ kDa}\text{-}1 \text{ }\mu\text{m}$), confirming that colloids can measurable contributions of BPs in both dissolved and colloidal phases, confirming that colloids can influence downstream transport and exposure (Si et al., 2019). The field patterns align with physiochemical properties moderate hydrophobicity for BPA ($\log K_{ow} \sim 3.3$) vs higher mobility for BPS ($\log K_{ow} \sim 1.6\text{-}1.7$) that shape sorption and travel distance (Corrales et al., 2015).

In lentic waters (reservoirs, lakes), longer residence times favor photochemical transformation and sorption/settling, often moderating water-column concentrations relative to inflows (Liu et al., 2021). Under natural sunlight and the presence of dissolved organic matter (DOM), indirect DOM-sensitized photolysis (via $^3\text{DOM}^*$ and ROS) dominates BPA loss, and rates are modulated by DOM quantity/quality and ions (e.g. nitrate/chloride) (Nathanael et al., 2024). Field-proximal experiments in coastal aquaculture waters show lower salinity can accelerate apparent BPA decay, consistent with ionic effects on radical speciation and DOM optics (Russo et al., 2022).

Estuaries and coastal seas integrate riverine loads with marine dilution, yet open seawater typically exhibits low ng L^{-1} BPA (and analogues) concentration, while marine sediments accumulate higher, time integrated burdens near anthropogenic nodes such as urban outfalls and working coasts. A marine meta-inventory and critical review converge on this picture: most seawater measurements fall in the low ng L^{-1} range (occasionally reaching the few $\mu\text{g L}^{-1}$ level near sources), and sediment concentrations are commonly an order of magnitude higher than surface waters at the same sites (Fabrello & Matozzo, 2022).

In groundwater, BPA is usually much lower than in the surface waters but spans a very wide global range, with reported values from 0.00009 to 228.04 $\mu\text{g L}^{-1}$; rare high outliers are linked to landfill leachate leakage, septic inputs, or rapid recharge pathways in vulnerable hydrogeology. These distributions imply that analogue specific sorption/retardation and aquifer properties shape mobility, with relatively more mobile BPS plausibly travelling farther when introduced even at lower mass than more hydrophobic analogues (Dueñas-Moreno et al., 2022).

At the drinking water interface, utilities frequently detect BPA, BPF and BPS in source waters at low to mid ng L^{-1} and typically reduce them to non-detected or single to few tens ng L^{-1} in finished tap water, depending on the treatment train (H. Zhang et al., 2019). Another study showed trace-level BPs in tap and bottled waters (ng L^{-1} scale) using SPE-LC-MS/MS, underscoring that treated waters are generally low but measurable. Treatment chemistry matters: free chlorine reacts rapidly with BPA with pH dependent kinetics, generating chlorinated intermediates whose persistence (hours) depends on residues and pH; monochloramine reacts more slowly, while ozonation/AOPs usually accelerate parent removal but form short-lived aromatic intermediates (Gallard et al., 2004).

3.5. Toxicological concerns of BPs

BPA has been phased out in many products and replaced with similar compounds like BPF, and BPS, which are still widely used and largely unregulated. These BPA substitutes are now frequently detected in the environment and in humans (Zhu et al., 2024). Most toxicological evidences is on BPA itself, which is associated with reproductive, metabolic, and immune problems (**Figure 3.4**) (Neri et al., 2024). BPA analogues have also been proven to be detrimental to human health and associated with cardiovascular diseases, cancer, obesity, fertility, and allergies (H. Wang et al., 2022).

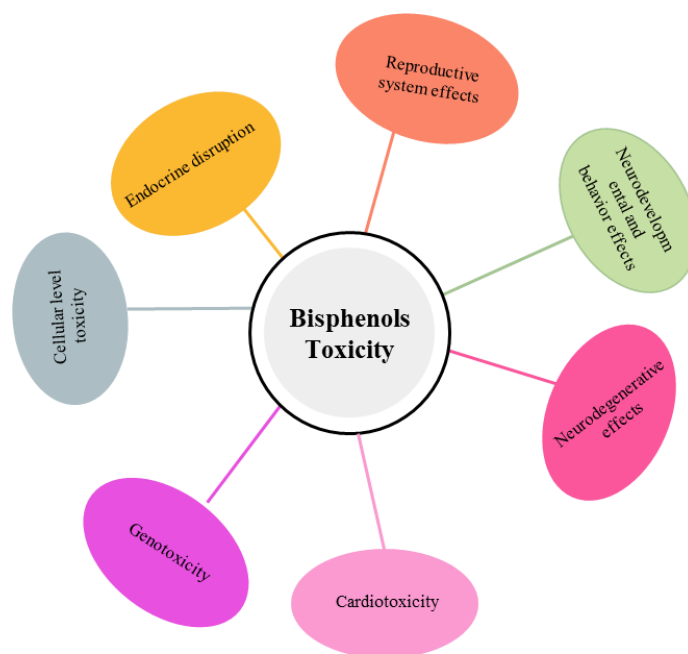


Figure 3.4. Toxicological effects of bisphenol A and its analogues.

3.5.1. Endocrine disruption

Bisphenols act as endocrine-disrupting chemicals and these compounds has been a major concern over the past decades, primarily due to their widespread use in consumer products and their potential health effects (Katchy et al., 2014; Siracusa et al., 2018). Across fish and amphibian models, BPs consistently trigger estrogen-responsive biomarkers and reproductive impairment, with clear structure activity differences among analogues. In zebrafish *in vivo* estrogen-reporter assays, BPAF and BPC elicit stronger ER dependent transcription than BPA, whereas BPS is typically weaker but still active at higher micromolar ranges; these patterns align with receptor pharmacology measured for the three zebrafish ERs and translate into vitellogenin estrogen-gene induction in embryos

and eleuthero-larvae (Karim et al., 2022; Pinto et al., 2019). Mechanistic studies in mammals and cells show that BPA and its analogues act through estrogen receptors, but not in the same way. BPAF and BPC bind ER α /ER β in a way that improves co-activator recruitment compared to BPA, while BPA itself binds very strongly to ER γ (IC₅₀ $\approx$ 13 nM), creating an extra low-dose estrogenic pathway. These receptor-level interactions are consistent with observed endocrine effects on steroid production, thyroid function, fat cell formation, and immune signalling in rodent and human cell studies helping explain why several analogues equal or exceed BPA's potency (Karim et al., 2022; Takayanagi et al., 2006).

3.5.2. Reproductive System effects

An assessment of the reproductive toxicity of these analogues in female rats unveiled that compared with the control group, both BPS- and BPF-exposed rats exhibited adverse morphological and histopathological changes in their ovaries, which manifested as a significant decrease in antral and corpus luteum follicles, whereas an increase in atretic and cystic follicles (Ijaz et al., 2020). The ovarian tissues of BPAF-exposed mice exhibited along with significantly modified follicle number and morphology. Pathologically, the number of primordial follicles significantly decreased, whereas that of cystic and atretic follicles significantly increased. The ovarian reserve (including primordial follicles, primary follicles, and secondary follicles) was significantly reduced compared with the control group (Yue et al., 2023).

In vivo investigations have shown a reduction in the quantity of ovarian follicles and the volume of antral follicles after BPS therapy. BPS modified the ovarian proteome and induced cytoskeletal damage in mature mouse oocytes, contingent upon dose (Nevoral et al., 2018). In *C.elegans*, 11 BPA analogues (1 mM) showed endpoint-specific toxicity: BPB, BPC, BPE, BPG, produced the highest embryonic/L1 lethality; BPAP, BPB, BPC, BPG caused developmental delay (fewer animals reached adulthood); BPAP, BPB, BPC2, BPG reduced egg production, and BPAP, BPB, BPC, BPG shortened lifespan, indicating several “BPA-free” substitutes are not safer (Ha et al., 2025). BPA exposure can also affect the normal operation of the mammalian reproductive system. Exposure to BPA may disrupt the normal functioning of the mammalian reproductive system. Exposure may diminish the quality and number of male germ cells, thereby resulting in aberrant development of the male reproductive system and harm to reproductive organs

(Long et al., 2025). Furthermore, the estrogenic properties of BPA may compromise the quality of female oocytes and might potentially contribute to the onset of PCOS (Y. Wang et al., 2017).

3.5.3. Neurodevelopmental and behavior effects

Studies in epidemiology indicate that prenatal exposure to BPA may modify the brain's structure and function, potentially resulting in neurodevelopmental disorders, including learning disabilities, sensory deficits, developmental delays, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (Buoso et al., 2025). Recent analyses have examined the relationships between BPF or BPS and ADHD symptoms across various developmental stages in children, specifically at ages 4, 6, and 8. Findings indicate that all bisphenols were linked to ADHD symptoms at age 6 (Kim et al., 2022). Furthermore, recent *in vivo* animal studies indicate that BPF and BPS, even at sublethal concentrations (1, 10, or 100% of their respective EC25), could present considerable risks to ASD development in humans. This underscores the necessity for a thorough evaluation of the developmental neurotoxicity effects associated with bisphenols (Bai et al., 2023; Musachio et al., 2024). Epidemiological studies examining emotional neurobehavioral issues have indicated a link between parental urinary BPA levels and the presence of depressive, anxious, and hyperactive behaviors in children, although some studies reported no such association (Minatoya et al., 2017; Musachio et al., 2024; Yolton et al., 2011). Conversely, the available data on alternatives to BPA are limited, indicating possible neurodevelopmental risks. Certain analogues, such as BPAP and TBBPA, have been shown to cause changes in the hippocampus associated with recognition behavior, sociability, and heightened anxiety levels. Meanwhile, other analogues, including BPS, BPF, BPE, BPB, BPZ, and BPAF, have demonstrated neurotoxic effects *in vitro*, pointing to neuro-inflammatory responses and microglial activation (Mhaouty-Kodja et al., 2024).

3.5.4. Neurodegenerative effects

Neurodegenerative diseases represent pathological conditions characterized by uncontrolled neuronal death across various brain regions. This phenomenon arises from changes in multiple cellular and molecular pathways, such as protein misfolding, mitochondrial dysfunction, oxidative stress, and neuro-inflammation, which have also been observed following exposure to bisphenol (Buoso et al., 2025). Furthermore, BPA

and its analogues disrupt estrogen and androgen receptors, suggesting that hormonal alterations resulting from bisphenol exposure may negatively impact the neuroendocrine system and promote the onset of neurodegenerative diseases (Rebolledo-Solleiro et al., 2020).]. Recent evidence suggests a potential connection between exposure to BPA and neurodegenerative diseases, particularly Alzheimer's disease (AD) and Parkinson's disease (PD) (as reviewed in (Rebolledo-Solleiro et al., 2020; Y. Xu et al., 2024)). Epidemiological studies indicate that BPA concentrations in liver and adipose tissue of AD patients are, respectively, 4.24-fold and 6.76-fold higher than those found in the age-matched control group (Manivannan et al., 2019). Furthermore, the plasma concentration of conjugated BPA was notably diminished in patients with PD, suggesting a compromised metabolic effect of BPA that leads to the binding of unconjugated BPA to albumin and protein disulfide isomerase (Landolfi et al., 2017). Various prospective cohort studies have indicated that exposure to BPA correlates with characteristic features of Alzheimer's disease and Parkinson's disease, including atypical behavior and cognitive functions (Braun et al., 2011; Perez-Lobato et al., 2016; Tewar et al., 2016). Data from *in vivo* and *in vitro* studies further indicate that exposure to BPA or its analogues, BPF and BPS, could significantly contribute to the heightened risk of these pathologies (Y. Xu et al., 2024).

3.5.5. Cellular level toxicity

The study by Xu et al. revealed that BPA and its analogues, particularly BPAF and BPA, significantly decreased mitochondrial activity and cell viability, with BPAF exhibiting the most pronounced effects. Exposure to these bisphenols led to increased mitochondrial reactive oxygen species (ROS) levels and elevated mitochondrial calcium concentration, indicating mitochondrial stress. Moreover, the BP induced apoptosis in the granulosa cells, highlighting their potential toxicity and suggesting that these analogues may not be safer alternatives to BPA (G. Xu et al., 2024).

The study mechanisms of toxicity caused by bisphenol analogues in human *in vitro* cell models investigate the toxic effects of BPAF, BPAP, BPE and BPP on human cell lines. The results showed that these analogues induce oxidative stress, mitochondrial dysfunction and altered calcium homeostasis, with BPAF and BPAP most toxic (Rifa et al., 2025). In mice, BPB (300 µg/kg bw/day) caused subclinical hepatotoxicity with reduced cholesterol, circadian rhythm disruption, and Tmem87b/Fkbp1a-linked

chemoresistance; maternal BPB produced offspring hepatomegaly and elevated transaminases, driving oxidative stress and lipid metabolism imbalance (Yue et al., 2025).

3.5.6. Genotoxicity

Recent observations have highlighted the genotoxic effect associated with pro-oxidative potential. The rise in oxidative stress correlates with heightened production of reactive oxygen species, the buildup of oxidation compounds (bio-macromolecules), and alterations in the activities of antioxidant enzymes (Ďurovcová et al., 2022). This behavior can manifest in organisms with both negative and positive estrogen receptors. Following the occurrence of intracellular oxidation, the likelihood of DNA damage, including both double- and single-strand breaks as well as oxidative damage, escalates. A further investigation indicates that BPA, when utilizing non-canonical estrogen as a receptor, associates with a membrane, with the receptor being linked to the G protein. Consequently, BPA has the ability to influence transcription factors and ion channels, leading to detrimental effects including mitochondrial stress, harm to the endoplasmic reticulum, and damage to the amyloid polypeptide in human islets (Banerjee et al., 2022). Comprehensive assessment of the safety of bisphenols A and its analogues based on multi-toxicity tests *in vitro* evaluates the cytotoxicity, endocrine disruption, and genotoxicity of various bisphenol analogues. The results showed that most bisphenols, particularly BPAF and BPAP, exhibit significant toxicity, including estrogenic activity and DNA damage, with BPAF being the most harmful (Wang et al., 2025).

3.5.7. Cardiotoxicity

Ma et al. analyzes the effects of BPA, BPS, BPF, and BPAF on human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as well as cardiac organoids. The findings reveal that exposure to 1nM BPA, BPS, and BPAF suppressed myocyte contractility, retarded contraction kinetics, and caused aberrant calcium transients in hiPSC-CMs, with BPAF exhibiting the most pronounced effects. In cardiac organoids, BPAF and BPA also resulted in suppressed contraction and abnormal calcium handling. Additionally, BPAF exacerbated hypertrophic phenotypes in endothelin-1-induced hypertrophic hiPSC-CMs and cardiac organoids, highlighting potential pro-hypertrophic cardiotoxicity (Ma et al., 2024). BPA induces changes in thyroid function by prompting the pituitary gland to generate thyroid hormones. These hormones oversee various functions within the body, including the metabolism of carbohydrates and lipids, as well

as the regulation of heartbeats. Indeed, exposure to BPA impacts the cardiovascular system [80] and may also play a role in the development of hormone-dependent conditions such as obesity and type 2 diabetes (Molina-López et al., 2023).

3.6. Analytical methods for BPs detection

BPs are widely present, appearing in numerous facets of human daily life, the environment, and even in human waste. To enhance the detection of BPs in both environmental samples and human biological systems, it is imperative to implement a range of effective and accessible detection methodologies without delay.

So far, the technology for detecting BPA has seen ongoing enhancements; nonetheless, traditional methods such as HPLC, liquid chromatography–tandem mass spectrometry (LC-MS/MS), UPLC-MS/MS, and gas chromatography–mass spectrometry (GC-MS) remain in use. LC represents the predominant analytical method, comprising over two-thirds of the referenced articles. This observation aligns with the understanding that overall LC facilitates the analysis of compounds across a broader spectrum of polarity and molecular volume, unlike GC, which requires molecules to possess inherent volatility or to be derivatized (Caballero-Casero et al., 2016; Neri et al., 2024). GC–MS continues as a valuable alternative for determination of mixtures of bisphenols but application to mixtures of diglycidyl ethers and chlorinated derivatives has been occasional. LC-MS/MS is generally preferred to analyze these compounds, because generic and fast methods for screening purposes can be developed, and the derivatization steps needed for a proper GC–MS analysis can be omitted (Danzl et al., 2009).

LC-MS/MS serves as the primary method for determining BPs and their derivatives in environmental waters due to its high sensitivity and compound-specific selectivity. This technique typically employs reversed-phase C18 columns, atmospheric pressure ionization (API) interfaces, and triple quadrupole analyzers functioning in multiple reaction monitoring (MRM) mode. In the realm of API interfaces, electrospray ionization (ESI) is often favored due to its superior sensitivity for bisphenols and their derivatives compared to atmospheric pressure chemical ionization (APCI) (Benijts et al., 2004; Goeury et al., 2019; Lee et al., 2023). Nonetheless, APCI has proven effective in identifying chlorinated derivatives in sewage sludge (Dorival-García et al., 2012; Dupuis et al., 2012), as well as in biological samples (Caballero-Casero et al., 2016; Jimenez-Diaz et al., 2010; Zhou et al., 2014).

Typical workflow use solid-phase extraction (SPE) often polymeric reversed phase or mixed mode sorbents to preconcentrate analytes from 100-1000 mL of water prior to LC-MS/MS, improving method LODs into the low ng L⁻¹ range (Caban & Stepnowski, 2020; Q. Wang et al., 2022). Selectivity in LC-MS/MS is enforced by monitoring two or more MRM transitions (quantifier and qualifier) per analyte and verifying ion-ratio tolerance, which is standard practice for endocrine –disruptor methods adapted from EPA 539.x style protocols (Goeury et al., 2019). Method performance in environmental waters is now routinely reported with LODs <10 ng L⁻¹ for BPA and several analogues when SPE is combines with isotope-dilution LC-MS/MS, although values vary by matrix and instrument (Chen et al., 2022; Q. Wang et al., 2022). For example, a surface water method targeting seven bisphenols via SPE-LC-MS/MS reported recoveries within 70-120 % and low ng L⁻¹ LODs across analyse, reflecting robust enrichment and selective MRM acquisition (Q. Wang et al., 2022).

Zhang et al. utilized ZnCl₂ as a catalyst in the synthesis of amino-functionalized poly (N-vinylpyrrolidone divinylbenzene) [P(NVP-DVB)]. The synthesized P(NVP-DVB) was then employed as an adsorbent for the detection of BPA in aqueous environments using HPLC and solid-phase extraction techniques. The recovery rate was recorded at 99.7%. This method shows effectiveness in both simulated and real water samples. Furthermore, established methods for detecting BPA involve the use of various composite materials. Cobalt nanoparticles and nitrogen-doped carbon nanomaterials were utilized on the surface of glass carbon electrodes for the detection of BPA. The enhancement of the device resulted in an impressive 98% recovery rate and exceptional electrocatalytic performance for BPA (Tang et al., 2023).

GC-MS, typically in electron-ionization (EI) mode remains highly competitive for bisphenols when combined with derivatization to improve volatility and chromatographic behavior (International organization of Standardization, 2009). Using an improved SPE-GC-MS (SIM) method with a matrix trap PSA layer, LODs of 1-50 ng L⁻¹ were achieved for 10 BPs in complex waters while maintaining recoveries of ~87-133% demonstrating strong sensitivity with tailored cleanup (Kiejza et al., 2022). Miniaturized or enhanced extraction formats paired with GC-MS such as ultrasound-assisted emulsification microextraction (USAEME), magnetic SPE, SPME, or non-column enrichment also reach low ng L⁻¹ LODs in environmental waters validated properly (Honeychurch, 2024; Kiejza et al., 2022; Koti et al., 2025; Viñas et al., 2010).

The analysis of bisphenols using GC–MS requires their derivatization through acetylation or silylation, followed by ionization via electron impact (EI) and the separation of ions in single quadrupole analyzers functioning in the single ion monitoring (SIM) mode. This method has maintained its popularity over the years, attributed to its exceptional capabilities and sensitivity. The derivatization of free hydroxyl functional groups in bisphenols results in distinct peaks, thereby enhancing their separation, sensitivity, and accuracy. The process of acetylation using acetic anhydride has been the predominant method for synthesizing bisphenol derivatives suitable for GC–MS analysis (Arar & Alawi, 2019; Jurek & Leitner, 2017; Kiejza et al., 2022). Silylation is widely utilized due to its rapid and complete reaction, resulting in thermally stable and highly volatile derivatives. The leading silylation reagents utilized for this application include N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), BSTFA with trimethylchlorosilane, and N,N-Methyl(trimethylsilyl)trifluoroacetamide (MSTFA) (Caballero-Casero et al., 2016). Alternative derivatization chemistries (e.g. pyridine-3-sulfonyl chloride or dansyl chloride) have been shown to boost MS signal and selectivity for phenolic endocrine disruptors including BPA by increasing response and enabling better chromatographic peak shapes, though they require rigorous control of reagent blanks (Regueiro et al., 2015; Viñas et al., 2010). Sensitivity for the determination of bisphenols by GC–MS is highly variable and it mainly depends on the level of analyte pre-concentration during sample treatment (Caballero-Casero et al., 2016).

Ultimately, method choice should reflect matrix complexity, monitoring objectives (screening vs. compliance vs. research), available QA/QC (e.g., isotope labelled standard), and the need to capture known/unknowns often leading to dual platform strategies where LC-MS/MS provides primary quantification and HRMS or GC-MS offers orthogonal confirmation (Baesu & Feng, 2024; Goeury et al., 2019).

4. Microplastics

4.1. Introduction

Plastic waste has increased by more than 20 times in the last 50 years, and it's likely to get worse until at least 2050 (Yang et al., 2023). The rise in global plastic output and post-consumer plastic trash is especially notable. The global metropolitan population is expected to generate about 6 Mt of solid trash everyday by 2025. Even with the current fraction of ~10% plastics in the solid waste stream, this corresponds to nearly 200 million ton of waste plastics—the 2002 global plastic resin production (Lebreton & Andrady, 2019).

Concerns about this kind of waste are growing, and most of them have to do with how long plastics stay chemically stable and how they break down into small pieces (5 mm) called Microplastics (MP) in the environment (through hydrolysis, photo-degradation and physical weathering) (Zeri et al., 2021). In recent years, MPs pollution has gained significant attention as scientists have discovered that these tiny pieces of plastic are present in every body of water on Earth, including ocean surface waters, seabed sediments, beaches, freshwaters, wastewater effluents, Arctic and Antarctic Sea ice, and even bottled water. Because of their ability to disperse pollutants, they are now a danger to ecosystems and human health (Martinho et al., 2022; Tang & Hadibarata, 2022).

Microplastics can be categorized into two main types: primary and secondary. Secondary MPs are the byproduct of bigger displaced plastics, but primary MPs are created on purpose, like in industrial resins (e.g., pellets for easier transport) or personal care products (e.g., abrasive particles in toothpaste or skin care). The breakdown of larger plastics into smaller particles caused by human activity or natural processes is a major contributor of MP in the environment (Han et al., 2020; Matjašič et al., 2023). Typical source of both primary and secondary MPs and their relative contributions are described in Figure 4.1.

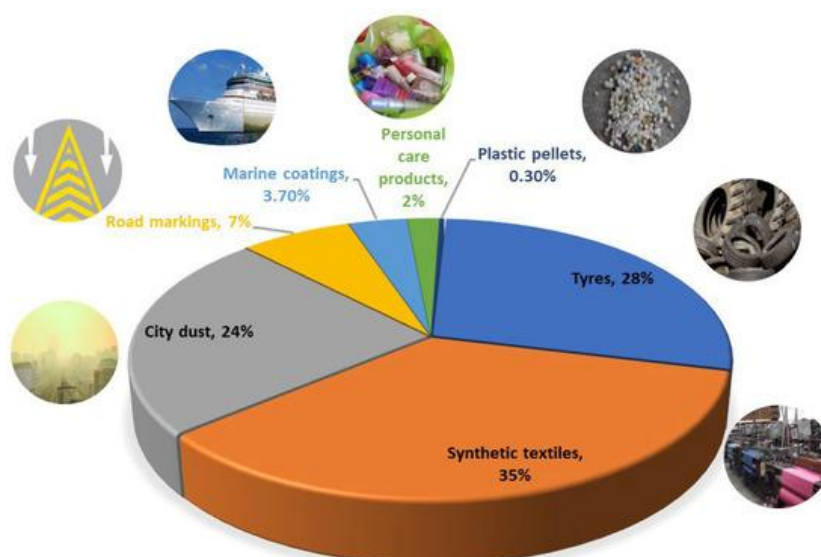


Figure 4.1. Relative contributions of major MPs sources (Pandey et al., 2022).

At the same time, MPs are no longer considered only an environmental pollutant “out there”. They have also been detected in human biological matrices, including lung tissue (Amato-Lourenço et al., 2021), intestinal tissue from colectomy specimens (Ibrahim, Tuan Anuar, et al., 2021), and even placenta (Ragusa et al., 2021), indicating that exposure is not only theoretical but measurable *in vivo* (Lu et al., 2018). MPs and nanoplastics (NPs) are also capable of altering gut microbial communities in experimental systems, shifting taxa and microbial metabolism in ways that may have health relevance (Tamargo et al., 2022). Together, these findings support the view that MPs are not only an environmental contaminant but also a measurable internal exposure with plausible biological consequences.

MPs vary greatly in particle concentration, polymer type, and size regardless of aqueous matrix. Analyses of various types of MPs are thus necessary from both a qualitative and quantitative standpoint, in order to understand their behavior and hazards, as well as to develop effective methods for their eradication. Recent years have seen significant advancements in analytical techniques for MPs (Umar et al., 2023). Extensive research indicates that the quantity and distribution of MP pollution require further investigation in order to develop effective remediation strategies (Yang et al., 2023).

4.2.MPs Concentration in WWTPs and surface water

MPs are an emerging class of contaminants that are causing significant concern in surface water and WWTPs (Ramos et al., 2024). MP concentration within wastewater treatment

plants can reach higher levels, ranging from $29.2\text{-}60.8 \times 10^3$ particles per kilogram (Chaudhary & Suthar, 2023; Contreras-Llin & Diaz-Cruz, 2024; Koyuncuoğlu & Erden, 2023; Magnusson & Norén, 2014). However, WWTPs currently lack dedicated infrastructure and treatment processes to effectively remove MPs (Ramos et al., 2024).

MPs enter the environment indirectly through WWTP effluents, together with their accompanying sludges or biosolids (Freeman et al., 2020). MPs are not only present in freshwater bodies, soils, and WWTPs, but they also serve as carriers, facilitating the movement of these contaminants through water runoff and wind towards significant reservoirs like oceans (Horton & Dixon, 2018). In a different study conducted in 2023 by Franco et al., (Franco et al., 2023), a comprehensive examination of sludge from seven separate WWTPs in Spain found MP concentrations varying from 0.02 ± 0.006 to 57.18 ± 20.69 particles/g. The most common polymers found in WWTPs were PP, acrylates, and PE, indicating a wide variety of polymer types and abundances. An important finding of the study was that sludge, when used as a soil supplement, becomes a major source of pollution in agriculture due to its ability to absorb MPs. SS requires adequate treatment prior to agricultural land; otherwise, its use as a fertilizer poses a considerable environmental risk, as emphasized by Chojnacka et al., 2023.

The concentration of MPs in WWTPs across countries is presented in **Table 4.1**, which gives a critical and global view of the issue and sheds light on its global scope.

Table 4.1: concentration of MPs in WWTPs across countries				
Matrix	Sample	Location	MPs Concentration	References
WWTP	Sewage sludge	Ireland	4196-15385 particles/kg	(Mahon et al., 2017)
WWTP	Sewage sludge	China	1565-56386 particles/kg dry sudge	(Li et al., 2018)
WWTP	Wastewater influent; Sludge; Effluent	Netherland	Influent: $68\text{-}910 \text{ L}^{-1}$ Sludge: $510\text{-}760 \text{ kg}^{-1}$ Effluent: $51\text{-}81 \text{ L}^{-1}$	(Leslie et al., 2017)
WWTP	Sewage sludge	Swedish	$16.7 \pm 1.96 \times 10^3 \text{ kg}^{-1}$	(Magnusson & Norén, 2014)

WWTP	Sewage sludge	USA	5 particles g ⁻¹	(Carr et al., 2016)
WWTP	Influent; Effluent; Dewatered sludge; Sludge filter cake	Northeast China	Influent: 126 ± 14 particles L ⁻¹ Effluent: 30.6 ± 7.8 particles L ⁻¹ Dewatered sludge: 36.3 ± 5.7 particles g ⁻¹ Sludge filter cake: 54.3 ± 6.2 particles g ⁻¹	(Jiang et al., 2020)
WWTP	Influent; Effluent	Paris, France	Influent: 260-320 × 10 ³ particles m ⁻³ Effluent: 14-50 × 10 ³ particles/m ³	(Dris et al., 2015)
STP	Influent; Effluent	Ganga River, India	Influent: 42±10 – 150±19 n L ⁻¹ Effluent: 3±1 – 22±5 n L ⁻¹	(Parashar & Hait, 2023)
WWTP	Influent; Effluent	Turkey	Influent: 4800 particles L ⁻¹ Effluent: 140 particles L ⁻¹	(Koyuncuoğlu & Erden, 2023)
WWTP	Influent	Victoria, Australia	Influent : Plant A: 55.3 ± 38.4 MP L ⁻¹ Plant B: 42.5 ± 20.1 MP L ⁻¹	(Fan et al., 2023)
WWTP	Influent; Effluent	Shaanxi, China	Influent: 73-98 particles L ⁻¹ Effluent: 8-16 particles L ⁻¹	(Yang et al., 2022)
STP	Influent; Effluent; Dewatered sludge; Sediment	China	Influent: 2.02-2.50 items L ⁻¹ Effluent: 0.27-0.48 items L ⁻¹	(Wan et al., 2022)

			Dewatered sludge: $3.719-6.949 \times 10^3$ items kg^{-1} Sediment: $1.84-8.23 \times 10^3$ items kg^{-1}	
WWTP	Influent; Effluent	Bangkok, Thailand	Influent: 77 ± 7.21 particles L^{-1} Effluent: 10.67 ± 3.51 particles L^{-1}	(Tadsuwan & Babel, 2022)
STP	Influent; Effluent	Tokyo, Japan	Influent: $1030 \pm 212 \times 10^3$ particles m^{-3} Effluent: $2370 \pm 513 \times 10^3$ particles m^{-3}	(Kameda et al., 2021)
WWTP	Surface water ; Fish ; Sediment	Korea	Surface water: 31.4 ± 28.5 particles m^{-3} Fish: 7.3 ± 7.3 particles/fish (upstream); 12.4 ± 17.9 particles/fish (downstream) Sediment : 493.1 ± 136.0 particles/kg (upstream); 380.0 ± 144.2 particles kg^{-1} (downstream)	(Park et al., 2020)
WWTP	Sludge	Chile	34 particles g^{-1}	(Corradini et al., 2019)
WWTP	Influent; Effluent	Shenzen, China	Influent: Plant-A: 86.3 particles L^{-1} Plant-B: 69.4 particles L^{-1} Effluent: Plant-A: 2.57 particles L^{-1} Plant-B: 0.60 particles L^{-1}	(Ren et al., 2023)
WWTP	Sludge	Southwest of Spain	$0.02 \pm 0.006 - 57.18 \pm 20.69$ particles g^{-1}	(Franco et al., 2023)

WWTP	Influent; Effluent	Guiyang, China	Influent: $32.5 \pm 1.0 \text{ n L}^{-1}$ Effluent: $5.0 \pm 0.4 \text{ n L}^{-1}$	(B. Zhang et al., 2023)
WWTP	Sludge	Ganga River, India	$29.2\text{-}60.8 \times 10^3 \text{ particles kg}^{-1}$	(Chaudhary & Suthar, 2023)
WWTP	Effluent	Spain	$60 \text{ to } 236 \text{ n kg}^{-1}$	(Contreras-Llin & Diaz-Cruz, 2024)

4.3. Detection and Identification of MPs (Spectroscopic method)

Plastics are used so extensively, many types of MPs enter aquatic systems, so reliable detection and identification are essential before removal. Screening often starts with simple physical observation (color, shape size), which is fast and low cost but mainly works for larger, visually distinct particles ($>500 \mu\text{m}$) and can miss smaller MPs. For this reason, chemical methods are required to confirm polymer type and structure. Both destructive and non-destructive are used including SEM-EDX, PLM, FTIR, Raman spectroscopy, and GC-MS, and combining multiple methods reduces the risk of overlooking certain MP categories (**Figure 4.2**).

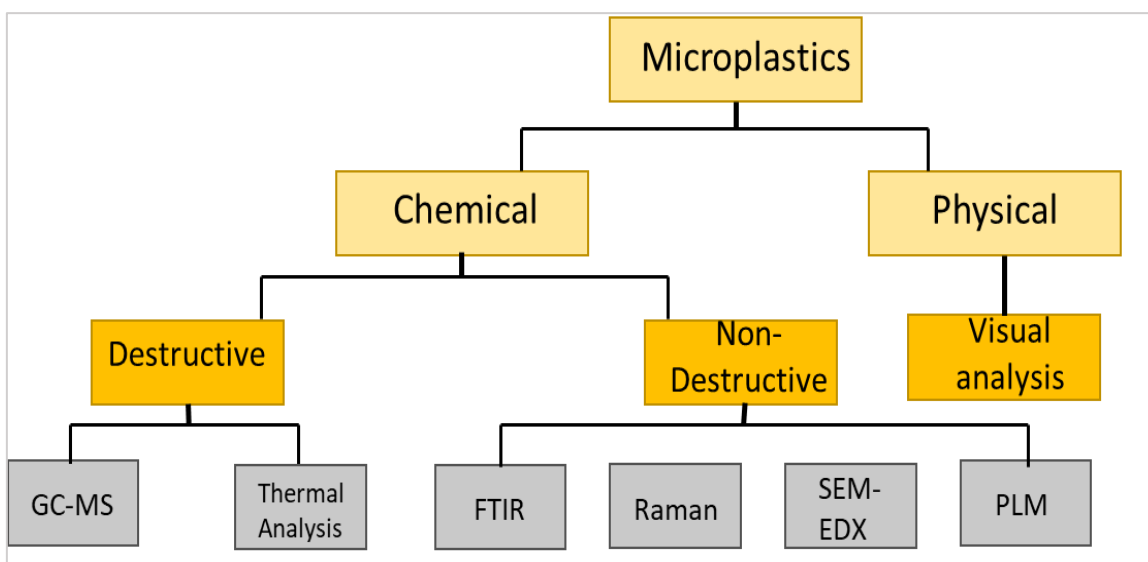


Figure 4.2. Methods for the detection and identification of microplastics.

4.3.1. FTIR Analysis

Fourier-transform infrared spectroscopy (FTIR) is a widely established, rapid, and highly reproducible analytical technique for identifying MP polymers in environmental samples. MPs are classified by matching acquired spectra with reference polymer libraries. Attenuated total reflectance FTIR (ATR-FTIR) is commonly employed for particles $>20\text{ }\mu\text{m}$, whereas micro-FTIR is used for smaller particles ($10\text{--}20\text{ }\mu\text{m}$) (Okoffo et al., 2022; Primpke, Christiansen, et al., 2020). Visual inspection is widely used as a presorting method for particles $>200\text{--}300\text{ }\mu\text{m}$ prior FTIR measurement ATR-FTIR is preferred in many studies for characterizing larger MPs isolated from various environmental matrices due to its low cost-effective, minimal sample preparation, and non-destructive reflective measurement mode (Primpke, Christiansen, et al., 2020). However, ATR may damage MPs owing to pressure exerted (Wu et al., 2019). Potassium bromide was used in several experiments to grind the bigger MPs so that transmission measurements can be done by diffuse reflection or ATR (Renner et al., 2018). Though these practices required higher time per particle approximately 3 min per particle Also these methods involve sample carrying to the laboratory. On the other hand, handheld FTIR system was also applied in some cases for direct analysis of MPs (Abidli et al., 2018) (Tang et al., 2019). Conversely, the handheld FTIR scheme is costly as compare to benchtop FTIR apparatus but these are usually restricted to characterize MPs within the size range of $300\text{--}500\text{ }\mu\text{m}$ (Primpke, Christiansen, et al., 2020).

Micro-FTIR method permits chemical analysis of greater zones of membrane with enhanced resolution by applying focal plane array (FPA) detection system (Bergmann et al., 2019; F. Liu et al., 2019; Primpke, Cross, et al., 2020; Simon et al., 2018). This type of approach was also comprehensively applied for analysis of MPs in combination with other systems like, ATR (Mühlschlegel et al., 2017; Schmid et al., 2018; Wagner et al., 2017), transmission (Compa et al., 2018; Liu et al., 2018; Lourenco et al., 2017), as well as reflection (La Daana et al., 2018; Lares et al., 2018). Although micro-FTIR is an efficient approach for analysis of MPs, it requires extended analysis period of about 20 h or more for each sample of MPs on a filter (Käppler et al., 2016; Primpke et al., 2017). Furthermore, there is also the possibility of sample degradation or damage during this analysis.

Reflection micro-FTIR requires adequate reflectance properties and is less suitable for small and colored particles, whereas transmission measurements may lead to total

absorption for large or highly dense MPs (Primpke, Christiansen, et al., 2020). Automatic FTIR system having FPA detector is mostly applied for quick acquirement of numerous spectra within a range by applying one single scan. Liu et al. employed a micro-FTIR system with a 125×125 mercury-cadmium-telluride based FPA detector to determine MP polymer types (F. Liu et al., 2019). Bergmann et al. (Bergmann et al., 2019) employed a micro-FTIR system equipped with a FPA detector to scan a range of 20×20 FPA (14.1 × 14.1 μm per pixel), enabling the detection of MPs as small as 11 μm within a 4.5-hour measurement period.

Löder et al., 2015, applied ten different types of filters with having different kind of materials, porosity as well as thickness to check their characteristics for FPA based detection system for MPs analysis with the help of micro-FTIR in reflection and transmission manner. Among these analyzed materials only two types of filters i.e., aluminum oxide and polycarbonate, were appropriate for characterization of MPs. The complications in using the other filters materials were their ranges of IR window, i.e., IR slide was also excessively narrow, IR features had great diffractive inaccuracy or absorbance ranges considerably greater than 0.5, bring about in imprecise IR spectra. Various substrates have been used for the detection of small MPs, including aluminum oxide and metal covered polycarbonate filters (Mani et al., 2019; Mintenig et al., 2019; Peeken et al., 2018; Primpke, Christiansen, et al., 2020), silicon membranes (Käppler et al., 2016), slides (Kunz et al., 2016; Wagner et al., 2017), compression cells (Cai et al., 2019; Frias et al., 2014), and windows prepared from infrared translucent or reflective substances (Pham et al., 2017; Primpke, Christiansen, et al., 2020). Nevertheless, water prevents FTIR-based MP analysis since its spectrum would obscure the target spectra (Primpke, Cross, et al., 2020).

4.3.2. Raman Spectroscopy

Raman spectroscopy is an alternative popular approach for identifying MPs. The procedure includes scattering the target particles by means of a laser of a certain wavelength. The elemental configuration of a sample is determined with the help of frequency of dispersed radiation from it. Due to the dispersed frequencies created by the sample, this systematic approach offers the ability to examine particles of considerably minor sizes with three-dimensional resolutions below 1 μm. (Imhof et al., 2016). But in most of these research, Raman spectroscopy wasn't used as the only way to identify MPs because of some factors like, enhanced background fluorescence, photo-degradation as

well as weathering, which caused the spectral variation. In many studies, it was proven that this approach worked well when applied with FTIR and electron microscopy together (Xu et al., 2019). So, only small portions of the filter can be analyzed at a time, which results in a lot of time consumption as well as intensive work. During the application of this technique there is another barrier which can limit its usage is spectral interferences. For example, Oßmann et al. (Song et al., 2021) found various essences, colorants and other complexes in bottled mineral water, such as CaCl_2 , MgF_2 , and SiO_2 , which result in enhanced background noise that drowned out the weaker Raman signals. Also, when identifying MP from samples, a supplementary wide-ranging library for identifying polymers should be accessible to that we can obtain better results (Xu et al., 2019).

Xue and coworkers proposed a method that combines spatial heterodyne spectrometer (SHS) as well as Raman spectroscopic (RS) technique to overcome the limitations of traditional MP sensing, including lower quality output and a lower signal-to-noise ratio (Xue et al., 2022). Firstly, this hyphenated technique was applied for quick and accurate detection of various plastic polymers as well as MPs in different samples. The primary prototype of this SHS based method and design catalog in the light of the implementation goal was developed in detail. The attained mercury lamp interferogram was used to develop the data inversion procedure, which comprised eliminating the baseline, correcting the flat field, and eliminating the toe. Mercury lamps were used in the SHRS system's setup and calibration processes. The precise practical specifications of the system, including its spectra range, resolution as well as Littrow wavelength, were also confirmed. The findings of sensing and comparing the PP Raman spectrum to a dispersive RS demonstrated that SHRS had a greater signal-to-noise ratio. The SHRS method was also used for the detection of MPs in various samples of polymeric plastics to show that it is a viable option (Xue et al., 2022).

In analyzing a large number of particles of MPs which are collected on developed filter paper, Song and co-workers discovered the most efficient method for identifying MPs to minimize false positives/negatives (Song et al., 2021). They examined and contrasted labor-intensive, automatic, and semi-automated identifying techniques. On the basis of all evaluation criteria taken into account in this research, the semi-automated method of ultrafast recording, spectrum description, and consequent human analysis for identified constituent part and fabricators proven suitable for MPs investigation. The as-prepared identifying approach increased sample representativeness while reducing operating time and eliminating human operator mistakes (Cabernard et al., 2018; Rochman & Hoellein,

2020). Due to the restricted numeral of spectral profile and absence of chemical image assessment, the completely automated approach was less accurate and efficient for identifying fiber and polymeric materials. The completely automated method yielded a disproportionately great amount of false positives in the worst-case development (Ly et al., 2022; Song et al., 2021). The systematic algorithm must include all variables and take into account false negatives/positives to produce reliable data in order to eliminate artifacts in automatic MP investigation. A complementary approach to completely automated and labor-intensive procedures that avoids the drawbacks of automatic methods and the drawbacks related to human limitations is semi-automatic inspection. Hence, RS was determined to be suitable for the study of small MPs, but it was time-consuming. Raman mapping could be a good sensing method for MP if the operative duration can be shortened. Generally, the FTIR as well as Raman techniques serve as complementary methods for MP detection and each has certain benefits and drawbacks (Cowger et al., 2020).

Table 4.2: Applications of spectroscopic techniques for the analysis of MPs.

Techniques	Type of MPs	Samples	References
Micro-ATR-FTIR	PE, PP, PET and PS based MPs	Aquatic sediments	(Morgado et al., 2021)
Micro-FT-IR with FPA detector	PE and PS base MPs	Sewage sludge	(Mintenig et al., 2017)
FT-IR with FPA detector	Acrylate, SAN, VAC-acrylic copolymer, PE, PP PE-PP copolymer, PEst, PS, PUR, PVC, EVA, PA, and PVA based MPs	WWTPs in Denmark	(Simon et al., 2018)
FPA-based reflectance micro-FTIR	PE, PP, PVC and nylon	Different stages of WWTP	(Tagg et al., 2020)
FTIR	PE, PP, PS and PET base MPs	two German WWTPs	(Roscher et al., 2022)

FTIR	PE, PET, PP, PA, PVC and PS base MPs	Southwest Europe WWTPs	(Menéndez-Manjón et al., 2022)
ATR-FTIR	PP, PA, PS, EPR and PE	Marine water samples	(M. Yan et al., 2022)
Micro-FT-IR with FPA detector	PA, PU and PS base MPs	Different type of food containers in China	(Zhou et al., 2022)
FTIR	PE and PS base MPs	Aquatic environmental samples	(Athulya & Chandrasekaran, 2023)
micro-Raman spectroscopy	PS, PE and PP based MPs	WWTP in Florence (Italy)	(Becucci et al., 2022)
micro-Raman spectroscopy	EVA, <u>PA6</u> , PC, ABS, SAN, LDPE, HDPE, PP, PE, PET, PVC and PE6	Effluent of WWTP of Germany	(Wolff et al., 2019)
Laser-Induced DUV Raman/Fluorescence Spectroscopy	PE, PP, PVC, PS, PC, PES, PE, PUR, PA and HDPE	Different environmental aquatic samples in Germany	(Post et al., 2021)
Laser-Induced DUV Raman/Fluorescence Spectroscopy	Pharmaceutical MPs i.e., carbamazepine, naproxen, tryptophan	WWTPs in Germany	(Post et al., 2022)
Raman imaging with smartphone	MPs and NPs released from clothes or trinkets.	Samples collected from Australia	(Luo et al., 2022)
Surface-enhanced Raman spectroscopy	PP, PE and PS based MPs and NPs	Different environmental	(Lv et al., 2021)

		aquatic samples in China	
Raman spectroscopy	MPs produced from PVC	Samples from Bunnings Warehouse, Australia	(Luo et al., 2022)

4.4. Other Techniques

4.4.1. Thermal methods of analysis

In contrast to spectroscopic methods, the thermal analysis approach is used to assess any form of variations in the chemical as well as physical characteristics of substances based on their temperature stability. By examining the thermal stability or degradation as a result of heat of polymers, thermal investigation can provide information regarding their composition (Mai et al., 2018). Generally, critical thermal examination approaches are less capable of determining size scattering than microscopic techniques (Elert et al., 2017; Huppertsberg & Knepper, 2018). The majority of MPs liquefy before they are pyrolyzed, hence TGA, in contrast to vibrational spectroscopy techniques, cannot offer statistics regarding size of the particles (Dümichen et al., 2015; Yu et al., 2019).

For quantifying MPs in environmental matrices, Dümichen et al., 2015 combines Thermal Extraction-Desorption Gas Chromatography Mass Spectrometry (TED-GC-MS). This approach eliminates the need for intensive pretreatment of samples, allowing larger sample masses to be processed. In this method, solid-phase absorbers are subjected to heat extraction via TGA, followed by desorption using GC-MS. The TED-GC-MS technique was found to be effective for identifying MPs in aquatic matrices from rivers and samples collected from biogas facility. By analyzing distinctive breakdown products, the method can identify and quantify a wide range of polymers. To ensure accuracy of results, replicate samples were carefully analyzed to minimize discrepancies caused by mass loss during heat treatment. TGA was conducted twice to profile the degradation patterns of the matrix as a result of heat exposure (Dümichen et al., 2017). Further studies used TED-GC-MS have demonstrated its suitability for analyzing MPs in water samples from urban sewage (Altmann, 2019), street runoff (Eisentraut et al., 2018), and residual waste. Additionally this method has been used to examine MP contamination in complex media such as urban waste and street overflow although research in this area is still in its

early stages (Becker et al., 2020; Goedecke et al., 2020). Differential scanning calorimetry (DSC) uses the melting of crystal-like areas and the resulting involvement of an endothermic indication to analyze MPs, whereas pyrolysis-based technologies such as TED-GC-MS and TGA-FTIR identify and quantify MPs through the use of breakdown products. A TGA-DSC work by Majewsky et al., 2016, is relevant to this brief review since it proved the quantitative determination of PE and PP in extracts obtained from wastewater runoff sections by means of the TGA-DSC technique. TGA data were utilized to give a summary of the degradation configurations as a result of heating of the pure polymeric materials as well as the wastewater mediums, while DSC signals were utilized for measurement and characterization by determining the range of the melting peak in proportion to the sample frame.

Table 4.3: Applications of thermal analytical techniques for MPs analysis

Technique	Heating conditions	MPs identified	Samples	References
DSC	0 to 280 °C	PS, PET, PP and LDPE	primary and secondary treatment sites of WWTP Columbia	(Hernández Fernández et al., 2022)
DSC	0 to 290 °C	PE, PA, PET, and PP	Industrial WWTP Germany	(Bitter & Lackner, 2020)
DSC	Heating up to melting	PE-LD, PE-HD, PP, PA6 and PET	Four German WWTP effluents.	(Bitter et al., 2022)
TGA/SDTA	25°C to 600°C	PE, PS, and PP	WWTP in Ruhleben, Berlin, Germany	(Goedecke et al., 2022)
TGA and DSC	75°C to 260 °C	Polyhydroxyalkanoates (PHA)	Samples from secondary sewage sludge USA	(Kumar et al., 2021)

TGA/MS-DSC	Up to different conditioning	P3(HA)	Municipal Wastewater, Athens, Georgia	(White et al., 2021)
TGA and DSC	30–600 °C	LLDPE, HDPE, PP, PS, PET, PA66 and SBR	Sewage sludge during purification	(Belone et al., 2021)
TGA/EGA	Up to 600 °C	PE-LD, PE-HD, PP, PE, PS, PA, PUR, PET, ABS, PC, EPDM, TWP etc.	Samples of Arctic region	(Primpke et al., 2022)
TGA and DSC	Heating up to melting	PE, PP, HDPE and LDPE	WWTP of Montevideo, Uruguay	(Rodriguez Chialanza et al., 2022)
(TGA-SPE/TDS-GC-MS)	25°C to 80 °C	Various type of degraded MPs	Textile WWTP	(Čurlin et al., 2022)

4.4.2. SEM applications

Visual examination and pre-sorting of MPs using optical microscopy, notably stereomicroscopy, has been widely employed in several research projects. MPs have been visually inspected to determine numbers and categorize them by size, shape, and color. MP fibers and particles have indeed been characterized by scanning electron microscopy, scanning electron in combination with electron diffraction, as well as energy-dispersive X-ray spectroscopy (Fries et al., 2013; Vianello et al., 2013). Based on microscopic analysis, MPs in WWTP sewage water and sediment samples can be classified as either microbeads, fibers, fragments, flakes, or films (Blair et al., 2019; Magni et al., 2019; Xu et al., 2020). The most prevalent color is white (60%) trailed by black (18%) and red (9%), while Some more studies, indicated that the proportions of MPs detected might comprise fibers (63%), fragments (15%), films (14%), flakes (7%), and microbeads (1%). The most common form of MP in samples taken from WWTPs is fibers, followed by pieces. Results for Ontario samples from the Guelph WWTP confirm the prevalence of white in the natural world (Canada) (Li et al., 2018; Mason et al., 2016). In conclusion,

more characterization is required because inspection is insufficient for differentiating fibers' chemical characteristics from those of other particles found in wastewater samples. Thermo-analytical methods like FTIR and Raman spectroscopy can be used to precisely identify and quantify MPs.

4.5. Chromatographic techniques

4.5.1. LC-MS analysis

Polycondensation polymers have been determined by liquid chromatography-mass spectrometry (LC-MS) analysis of their low molecular weight derivatives. PET polymers were quantified by LC-MS/MS of *p*-phthalic acid obtained by alkaline hydrolysis with KOH and deuterated surrogate as internal standard. The method was applied to study the “release of polyester fibers clothes through washing and in two WWTPs (Y. Zhao et al., 2021).

Just a small number of authors have explored the possibility of MS for the study of MPs as well as NPs. Polystyrene (PS) MPs and NPs in waters sources were studied using a variety of methods, including matrix-assisted laser desorption ionization (MALDI) in combination with time-of-flight mass spectrometry (TOF-MS), liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), as well as ambient ionization methods like desorption electrospray ionization (DESI). An LC-HRMS approach was developed for the quantifiable study of MPs in aquatic systems; this method uses an atmospheric pressure photoionization source (APPI) to ionize the sample under adverse conditions. To enhance the chromatographic separation, an advanced polymer chromatographic (APC) column was utilized with an isocratic toluene mobile phase. Efficient removals of 60% and 70% have indeed been measured for river and ocean coastal samples, correspondingly, with an ILOD of 20 pg L⁻¹ and method limits of detection as well as quantification of roughly 30 pg L⁻¹ and 100 pg L⁻¹, correspondingly. The method was verified on both untreated and treated saltwater samples (Tian et al., 2021).

Table 4.4: Applications of LC/MS technique for the analysis of MPs.

Technique	MPs analyzed	Samples	References
LC-MS/MS	Mass of PET was determined	Laundry wastewater, influents, and effluents of WWTP	(Tian et al., 2021)
HPLC-ESI-MS/MS	PET, PC, and their monomers of terephthalic acid and BPA were analyzed	Sewage sludge	(J. Zhang et al., 2019)
LC-UV	Quantification of PET MPs and NPs	Sand, indoor dust and liquid sludges	(Tian et al., 2022)
LC-MS/MS	Nylon 6	detected in indoor dust, sludge, marine sediment, freshwater sediment, fishery sediment	(Peng et al., 2020)
LC-MS/MS	PET and PS were determined	Surface water of Center Hill Lake and WWTP of Cookeville	(Onwuchekwa, 2022)
LC-MS/MS	Masses of PA, PET, PP, PSF, and PU were determined	Sediments of a man-made lake	(X. Yan et al., 2022)
LC-MS/MS	Analysis of PE	Water-plant-sediment	(Yu et al., 2022)

4.5.2. Pyrolysis coupled with gas chromatography-mass spectrometry

Pyrolysis (from the Greek pyro = fire, lysis =breakdown) is the thermal decomposition of a substance in an inert or oxygen free (or very low oxygen) environment. Under pyrolysis, chemical bonds (C-C, C-O, C-N, etc.) cleave, yielding smaller volatile or semi-volatile fragment (monomers, oligomers, radicals), residual char, and gaseous products (e.g. CO, CO₂, light hydrocarbons) depending on conditions (temperature, heating rate, residence time, atmosphere) (Santos, 2026; Seeley & Lynch, 2023).

Pyrolysis-Gas chromatography-mass spectrometry (Py-GC-MS) is an analytical hyphenated technique in which samples is thermo-decomposed (pyrolyzed) into smaller volatile fragments, which are then separated by GC and finally detected/identified by MS (Dumont et al., 2025; Lykkemark et al., 2024; Peel et al., 2025; Seeley & Lynch, 2023). Pyrolysis itself, does not provide analytical data unless it is associated with some kind of measurement process. That is, it can be considered a kind of sample pre-treatment prior to instrumental analysis. In this context, Py-GC-MS is the most convenient and complete analytical method, combining thermal degradation at high temperature to products separation and chemical identification (Primpke, Christiansen, et al., 2020). Py-GC-MS operates by subjecting polymeric materials to high temperatures ranging from 550 to 1400°C, under an inert atmosphere within a pyrolyzer, leading to thermal decomposition (pyrolysis) of the sample. The resultant degradation products (pyrolyzates) are then separated via GC and identified based on their molecular profiles using MS. Characteristic pyrolysis fragments distinctive for each polymer enable fast, reproducible identification and quantification in a single analytical run (Dumont et al., 2025; Kusch, 2019; Santos, 2026).

4.5.2.1.GC-MS application for analysis of MPs

Py-GC-MS has been applied to analyze a wide range of sample types, including sediments, soil, water, air particles, biota, and human tissues (La Nasa et al., 2020). The focus here is on wastewater samples, as their matrix closely resembles that considered in this thesis.

This technique was employed to evaluate the variety of MPs in a sewage treatment plant that releases MPs into a stream channel. Throughout the year, GC-MS was capable of identifying the significant presence of a wide variety of polymers as well as their respective decomposition products (Roscher et al., 2022). A pressurized pump-assisted

steel-based cascading filtering apparatus with filters that are approximately 100, 50, and 10 microns in size was created to remove MPs. This MP extraction technique is then accompanied by MPs characterization in Py-GC-MS. For an effective pyrolysis procedure, they added a platinum filament, which allowed them to operate at temperatures around 550°C and 1300°C for 8 minutes. For MPs made of PS and PE, their approach was effectively verified. Using GC-MS as one of the key techniques, MPs and a plastic ingredient called Di 2-ethyl hexyl phthalate (DEHP) were found and characterized in a wastewater treatment center based on their size, particles density, shape, and color (Funck et al., 2020). Their research indicates that WWTP do not properly eradicate MPs since they still found MPs (2.419×10^7 particles/day) in the effluents yet after treatment of wastewater. Ibrahim et al. recently used ATR- FTIR as well as Py-GC-MS to study the dispersion of MPs in the surface water and sediments of the Setiu Wetland in the South China Sea (Ibrahim, Hamzah, et al., 2021). According to their findings, MPs are present in dry sediments and groundwater in concentrations of 5.97 and 0.36 items/g, correspondingly. While using GC-MS as one of the analysis techniques to describe the MPs, Takdastan et al. (Takdastan et al., 2021), and Ibrahim et al. (Ibrahim, Hamzah, et al., 2021) have not elucidated on their experimental protocol. Zhang et al. (Zhang et al., 2020) created a brand-new, specially designed handheld Py-MS in which a small pyrolyzer breaks down MPs before investigation in the portable MS, skipping difficult sample extraction stages. The technique has been verified for MPs of variety of plastic polymeric materials.

Table 4.5: Applications of GC/MS base techniques for the analysis of MPs

Technique	MPs identified	Samples	References
Pyr-GC/MS	PE, PP, PET, PS, PVC, PC, and PMMC	German Bight waters	(Dibke et al., 2021)
Pyr-GC/MS	PE, PA, PET, PP and PS	Raw and treated drinking water	(Gomiero et al., 2021)
Pyr-GC-MS	PE, terephthalate, nylon-6, and polyacrylonitrile	Textile laundry wastewater	(S. J. Lim et al., 2022)
Pyr-GC-MS	PE, PP and PS	River water and sediment	(Scherer et al., 2020)

Pyr-GC-TOF-MS	PVC, PA, PET, and PE	Wastewater samples	(Vilakati et al., 2021)
(TED)-GC/MS	PS and PE	Wastewater samples	(Funck et al., 2020)
Pyr-GC-MS	PVC, PP and PE	Western Lake surface water	(Hendrickson et al., 2018)
Pyr-GC-MS	PMMA, PP, PS, PE, PET, and PA	WWTPs in China	(Xu et al., 2023)
Py-GC-MS	PE, PP, PET, PVC, PMMA	Municipal WWTPs	(Okoffo et al., 2023)
Py-GC-MS	PE, PP, PS, PET, PVC, PMMA and PA	Two German WWTP	(Roscher et al., 2022)
Double-shot Py-GC/MS	PS, PC, PMMA, PP, PET, PE, and PVC	Treated sewage sludge	(Okoffo et al., 2020)
GC/MS	DEHP	WWTP	(Takdastan et al., 2021)
Pyr-GC-MS	PS and PE based tiny MPS	WWTPs	(Kook & Park, 2022)

4.5.2.2. Matrix effect in Py-GC-MS

“Matrix effect” refers to interferences or signal perturbations arising from coexisting substances in the sample matrix (i.e. non-target compounds) that affect the efficiency of pyrolysis, chromatographic separation, ionization, or detection (Santos et al., 2023). Environmental samples often contain a mix of plastics and natural organic/inorganic materials. Pyrolysis of these mixtures produces a wide array of volatile compounds, some of which can overlap with or mask polymer specific markers (Cho et al., 2023; Seeley & Lynch, 2023). Historically it has been challenging to quantify using pyrolysis, and consequently it has predominantly been used for qualitative and semi-quantitative work (Akoueson et al., 2021; Renner et al., 2018; Silva et al., 2018). The process is inherently complex as a wide range of pyrolytic products are produced in almost all environmental (Fischer & Scholz-Böttcher, 2019). samples This results in matrix effects that can heavily impact the peak areas of the target compounds. For example, internal surfaces can become

coated with heavier breakdown products that condense within the instrument, affecting the way that other pyrolytic products interact with these surfaces (Fischer & Scholz-Böttcher, 2019; Fischer & Scholz-Böttcher, 2017). Generated artefacts may also coelute or affect the volatility of other pyrolysis products (Groenewold et al., 2017). Another major factor affecting the reliable analysis of complex samples is variability in pyrolytic breakdown (Groenewold et al., 2019; Moldoveanu, 2001). Small changes in the analytical conditions can affect the composition of pyrolytic products, making quantification challenging and necessitating time-consuming optimization of a given pyrolysis set-up, alongside external calibration (Bart, 2001). Calibration curves generated from pure polymers may not accurately reflect response in real samples unless matrix-matched standard or internal standards are used (Lykkemark et al., 2024; Peel et al., 2025). Sample loss or incomplete decomposition due to matrix complexity may lower recovery rates and result in underestimation of MPs (Lykkemark et al., 2024; Okoffo & Thomas, 2024). Background contamination, such as airborne plastics or lab ware derived MPs can severely distort mass balance results or introduce non-native polymers. Strict quality control and procedural blanks are necessary (Santos et al., 2023).

To mitigate matrix interferences, in the literature the following approaches were described:

- Rigorous pre-treatment (digestion, oxidation, purification) to remove as much non-plastic organic residue as possible while preserving polymers (Fischer & Scholz-Böttcher, 2017; Gomiero et al., 2019; Kirstein et al., 2021; Silva et al., 2018).
- Thermal desorption/mild pre-pyrolysis steps (double-shot Py-GC) to purge volatile or semi-volatile organics adsorbed on the matrix (background organics) that otherwise would co-elute or interfere in main pyrolysis run (Seeley & Lynch, 2023).
- Use of internal standards/isotopically labelled pyrolysis standard added before pyrolysis to normalize signal suppression or variation (Jing et al., 2025; Peel et al., 2025).
- Matrix-matched calibrations/standard addition i.e. calibrating in a blank matrix similar to the sample to compensate for matrix suppression/enhancement (Lykkemark et al., 2024; Wiesner et al., 2021; Willner et al., 2023).

4.5.2.3. Sample pretreatment

Py-GC-MS is recognized for its need for minimal sample preparation. Although this holds for individual plastic fragments that can be sliced and pyrolyzed directly, the majority of environmental samples necessitate some form of preparation. Preparing samples for Py-GC-MS is essential to (1) enhance the concentration of plastics in a sample for analysis and (2) reduce the interference from the sample matrix (3) minimize interference of the sample matrix (Seeley & Lynch, 2023). Pyrolysis also requires less sample pre-treatment than Fourier-transform infrared spectroscopy (FT-IR) and Raman analysis with protocols relying less heavily on the manual selection of particles, thereby reducing the potential for human error (Peel et al., 2025).

The digestion process is the key procedure during MPs extraction and is of great importance to the extraction efficiency (Nuelle et al., 2014; Wang WenFeng et al., 2017). Fenton's reagent (Hurley et al., 2018; X. Liu et al., 2019), acid and alkaline (Li XiaoWei et al., 2020; Roch & Brinker, 2017), and enzymes (Mintenig et al., 2017; Wang et al., 2020), of which 30% H₂O₂ is one of the most commonly used methods for removing undesirable organics without changing the properties of the extracted MPs (Nuelle et al., 2014; Tagg et al., 2015). Fenton's reagent can effectively degrade organic components in a shorter period of time (Alvim et al., 2020; Hurley et al., 2018), but various factors, such as the concentration of organic matter, pH, and the ratio of Fe²⁺ to H₂O₂ restrict the extraction efficiency. However, the reaction temperature should be controlled and kept between 20 and 40 °C, avoiding too harsh oxidative conditions. In addition, iron sludge tends to interfere with MPs separation during extraction. Considering acidity and alkalinity during digestion, the concentration of solutions should be given special attention because the characteristics of extracted MPs are altered under extremely low or high pH conditions (Alvim et al., 2020; Hurley et al., 2018; Li XiaoWei et al., 2020; Okoffo et al., 2019).

Enzymatic digestion represents a novel method for the purification and extraction of MPs from environmental samples. Nonetheless, this process requires more time, and it is essential to meticulously regulate the enzyme storage and reaction temperature to preserve their activity, which restricts its use for digestion (Okoffo et al., 2019; Wang et al., 2020). Traditional digestion techniques are more effective for extracting microplastics from marine, freshwater, and aquatic organisms. However, when these methods are used on wastewater treatment plant samples, they typically require more time, a greater amount

of digestion reagents, or yield lower recovery rates of microplastics. Therefore, it is essential to create an effective method for extracting MPs from WWTP samples. Nonetheless, the existence of extracellular polymeric substances in sludge could impede the extraction of MPs. Excessive digestion reduces the efficiency of MPs extraction, necessitating the optimal dissolution of organics. Consequently, the correct application and concentration of digestion reagents are crucial and must be thoroughly investigated (Z. Zhang et al., 2023).

4.5.2.4. Internal standards

Quantifying MPs by Py-GC-MS is challenging (Lykkemark et al., 2024): external calibration achieve strong linearity in some systems, but variability in pyrolytic breakdown (Groenewold et al., 2019; Moldoveanu, 2001), interaction with internal surfaces (Fischer & Scholz-Böttcher, 2019), ion source conditions (Unice et al., 2012), and matrix derived artefacts often undermines robustness (Bocchini et al., 1997); using internal standard help correct instrument and matrix variability and has been adopted in only few MP studies so far.

In pyrolysis, the internal standard should behave similarly to target yet be absent from samples (M. Wang et al., 2017); polymeric IS have been used, but simpler molecules are often preferable to avoid spectral complexity, and insoluble or highly polar standard can be impractical without thermochemolysis (He et al., 2020; Lauschke et al., 2021; Unice et al., 2012). Employing internal standards can effectively address numerous challenges related to calibrant/sample preparation and instrument variability. Fischer and Scholz-Böttcher reported (Fischer & Scholz-Böttcher, 2019), improved calibration using a combination of internal standards: 9-dodecyl-1,2,3,4,5,6,7,8-octahydro anthracene, anthracene-d10, androstane, and cholic acid methyl ester (each 0.02 mg mL⁻¹ in n-hexane), collectively termed ISTDOPY. These serve as analogues of the marker compounds, representing aliphatic, planar aromatic, and non-polar aromatic compounds, rather than parent polymers. In certain instances, 9-tetradecyl-1,2,3,4,5,6,7,8-octahydro anthracene was incorporated to simulate the methylation of acid groups throughout the thermochemolysis process. The authors identified the optimal internal standard for each polymer through calibration curve fitting, observing a reduction in standard deviation from 100 to 6 µg with the use of internal standards. Previous studies have effectively utilized this ISTDOPY solution (Dibke et al., 2021; Fischer et al., 2019; Primpke, Fischer,

et al., 2020) or solely anthracene (Nakano et al., 2022). utilized 1,3,5-tri-tert-butylbenzene as an internal standard for the quantification of polystyrene through offline pyrolysis, while David et al. (David et al., 2018) employed DL-Cysteine as an internal standard for TGA measurements of PET. Additionally, Windt et al. (Windt et al., 2011) employed fluoranthene as an internal standard for the quantification of polypropylene through off-line pyrolysis.

A noteworthy strategy involves employing stable isotope labeled synthetic polymers as internal standards. The primary benefit stems from their resemblance to the analytes, particularly in terms of their solvation and pyrolysis characteristics. Unice et al. (Unice et al., 2012) presented the application of deuterated polymers, specifically poly(isoprene-d8), poly(butadiene-d6), and poly(styrene-d8), as internal standards for quantifying rubbers derived from tire tread particles. Poly(styrene-d5)(PS-d5) has also been utilized as an internal standard for the quantification of MP (Dierkes et al., 2019; Goedecke et al., 2020; Goßmann et al., 2021; Scherer et al., 2020). The application of alternative stable isotope (like ^{13}C) labeled synthetic polymers as an internal standard for microplastic analysis remains unreported. Stable isotope labelled standards are frequently utilized in GC-MS and LC-MS analysis; however, deuterated standards, in particular, are susceptible to potential exchange between deuterium (D) and hydrogen (H) atoms (Stokvis et al., 2005). Poly(styrene-d5) is commonly utilized as an internal standard for mass spectrometry quantification; however, it is prone to hydrogen-deuterium exchange during pyrolysis, which may compromise the accuracy of both identification and quantification. Poly(4-fluorostyrene) (PFS) presents a compelling alternative, as it is not affected by exchange reactions. The results from both standards were similar in calibrating polypropylene, polyethylene, and polystyrene MP; however, utilizing PFS allowed for a broader concentration range to be addressed. Minor interferences between both polymers and components of the natural organic matrix were observed (Lauschke et al., 2021).

4.5.2.5. Pyrolysis markers of synthetic polymers for the analysis of MPs

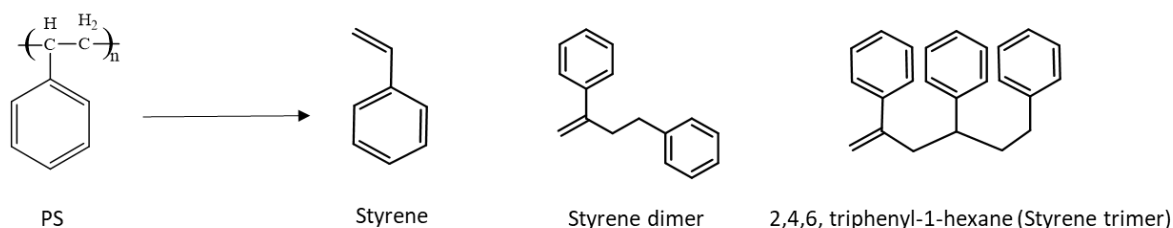
During pyrolysis, pyrolyzates can be directly formed (primary pyrolysis products) or secondary reactions can occur between, producing secondary pyrolysis products. Several types of reactions can take place, the most common are eliminations and rearrangements, but also oxidations, reductions, substitutions, or additions also are possible (Moldoveanu, 2005).

Thermal analysis and analytical pyrolysis methods are based on the thermal decomposition of polymers or polymer mixtures and on their characterization through the analysis of the pyrolyzates (La Nasa et al., 2020). Pyrolytic degradation of the polymers has been discussed extensively (Tsuge et al., 2011). PE and PS as the target polymers studied for their Impact on gut microbiota in an *In Vitro* colon model. PE are characterized by a series of hydrocarbons from polymer decomposition. For PE, pyrolysis yields a series of n-alkane, alkene, and alkadiene triplets; in the C₁₁ to C₃₃ range, formed by random scission (Scheme 4.1) of the polymethylene carbon backbone followed by hydrogen abstraction, double bond formation and beta scission.



Scheme 4.1. Main thermal degradation products of PE.

The major pyrolytic products for polystyrene (PS) is identifiable by styrene monomer, dimer, and trimer (Scheme 4.2) (Kusch, 2024; Rauert et al., 2021; Rødland et al., 2022).



Scheme 4.2. Main thermal degradation products of PS.

4.6.Conclusion

Each method has its own advantages and limitations. Vibrational spectroscopy and SEM provide concentrations based on the number of particles, which can be influenced by the range of particle sizes analyzed. Thermal and chromatographic methods are mass-based; however, they are destructive and do not offer morphological details. A key challenge in MP reporting is ensuring the comparability of data.

In summary, the investigation of MPs in sewage sludge (WWTPs) establishes a foundation for exploring similar analytical challenges in a biomedical context. The next chapter transition (Section III, Chapter 4) to an *in vitro* human colon model, where the

task of detecting MPs faces a comparable obstacle: a complex, organic-rich matrix that can obscure analytical signals. Residual biological matter in both sludge and colon fermentation media can induce severe matrix interference. For example, by causing fluorescence or infrared absorption that masks polymer signatures in spectroscopic analyses, and can likewise generate extraneous pyrolysis fragments that confound Py-GC-MS detection. To surmount these difficulties, both systems employ parallel methodologies. Each relies on the same methods to break down organic matter by appropriate sample pre-treatments. This conceptual and methodological continuity not only facilitates the transition from an environmental sludge matrix to the human colon model, but also underscores that the core analytical challenge isolating trace plastics from an organic-rich matrix remains fundamentally the same.

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II. Aims

This thesis addresses the urgent need for advances analytical methods to monitor the ubiquitous presence of emerging contaminants, specifically UV filters, bisphenols, and microplastics, across environmental and biological matrices. These contaminants have significant environmental and health implication due to their persistence, bioaccumulation potential and toxicological effects. This research aims to develop and optimize novel analytical techniques to improve the detection, quantification, and understanding of their environmental transformation and biological impacts.

Despite growing regulatory concern, significant analytical challenges persist in detecting these contaminants at trace levels in complex matrices. Furthermore, understanding their environmental transformation such as photoisomerization of UV filters, and biological impacts, particularly microplastics-microbiome interactions in human relevant models, remains critically underexplored. The development of sensitive, selective, and sustainable analytical methodologies is therefore imperative for comprehensive exposure assessment, environmental monitoring, and evidence based risk evaluation.

This thesis addresses these challenges through the following aim and objectives:

- Implementation of solid-phase extraction coupled with gas chromatography-tandem mass spectrometry for simultaneous determination of eleven UV filters and transformation products in Adriatic seawater, achieving sub ng L⁻¹ detection limits and comprehensive environmental monitoring (Section III, Chapter 1).
- Systematics evaluation of *E*-ethylhexyl methoxycinnamate (EHMC) photoisomerization kinetics under UV radiation in aquatic (swimming pool water, seawater and river water) and cosmetic matrices (sunscreen cream formulation, hair oil, lip balm) using optimized SPME-GC-MS analysis to assess photoprotective efficacy and environmental fate (Section III, Chapter 2).
- Optimization of *in vial* acetylation derivatization coupled with SPME-GC-MS for nine bisphenol analogues (BPA, BPAF, BPF, BPE, BPG, BPB, BPA, BPM, BPC2) in drinking, surface, waste and seawaters. Application of green chemistry matrices (AGREEPrep, BAGI) to validate method sustainability while achieving excellent analytical performance. Subsequently application to environmental samples for occurrence assessment and risk characterization (Section III, Chapter 3).

- Employing Py-GC-MS protocol to qualitatively and quantitatively determine PE and PS MPs in samples from an *in vitro* human colon model over the course of the fermentation process. Within an interdisciplinary collaboration, the specific objective was to monitor how controlled exposure to PE and PS MPs alter gut microbial community structure and metabolic activity. Building on this analytical and microbial characterization, we further sought to assess the potential health relevance of these shifts by linking microbiome dysbiosis and metabolic alterations to plausible disease pathways. (Section III, Chapter 4).

In conclusion this research aimed to integrate state-of-the-art analytical techniques including miniaturized sample preparation methods (SPE, SPME), green chemistry principle, and advanced mass spectrometry platforms (GC-MS, GC-MS/MS, PY-GC-MS) to develop and optimize sensitive, sustainable, and reliable methods for monitoring UV filters, bisphenols, and microplastics. The findings will significantly contribute to enhance environmental monitoring capabilities, exposure assessments, and strengthening regulatory frameworks for these widespread contaminants. Ultimately, this study aims to provide vital data for that will inform public health protection strategies, promote ecosystem preservation, and support evidence-based policymaking for mitigating the risks associated with emerging contaminants.

III. Results and Discussion

1. Quantification and Characterization of UV Filters in Coastal Seawaters of Adriatic Beach Environments

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1.1. Introduction

The UV filters contained in sunscreens and personal care products are widely used to protect the skin from harmful UV radiations preventing premature skin ageing, sunburn, and skin cancer, however, they have raised controversies for their potential negative effects (Breakell et al., 2024). Their widespread occurrence in aquatic environments accompanied by their potential toxicity has led to recognise UV filters as Contaminants of Emerging Concerns (CECs) (Gandar et al., 2025). Despite these concerns, environmental quality guidelines are still lacking due to gaps in environmental risk assessments, thus it is growing the interest in studying their distribution in water systems.

UV filters are regulated in cosmetics by European Regulation, which limits their concentrations (with some being prohibited and other restricted) (European Union, 2009). The European Union (EU) currently allows 27 UV filters, with concentrations limit ranging from 2% to 15% (Cadena-Aizaga et al., 2020). The global production of UV filters, including avobenzone (AVO), benzophenone-3 (BP-3), (*E*)-2-ethylhexyl methoxycinnamate (*E*-EHMC), octocrylene (OCR), and 4-methylbenzylidene camphor (4-MBC), has significantly increased, from over 10,000 tons in 2005 to 26.9 million tons by 2020 (Brausch & Rand, 2011). Among these, AVO and OCR are the most commonly used, appearing in over 40% of sunscreen formulations (Sun et al., 2024). Until 2015, water policy lacked references to UV filters, but that year, *E*-EHMC a widely used UV filter, was added to the EU's Watch list for monitoring substances in water (Mokh et al., 2022). Thereafter EHMC was removed since the monitoring data quality was good enough to perform the risk assessment, while three UV filters were candidate, namely OCR, AVO and BP-3 (Gomez Cortes et al., 2022).

E-EHMC is of particular concern due to its photo instability, undergoing photoisomerisation to (*Z*)-2-ethylhexyl methoxycinnamate (*Z*-EHMC) under sunlight, a transformation product associated with greater toxicity potential than its parent *trans* isomer (Nečasová et al., 2017; Sharma et al., 2017). In light of recent SCCS evaluations flagging *E*-EHMC for its potential endocrine-disrupting effects (Bernauer et al., 2025),

investigating both the parent compound and its transformation product is essential for comprehensive environmental risk assessment.

The environmental impacts of UV filters have raised growing concerns, leading to more studies in recent years (Brausch & Rand, 2011). These compounds enter aquatic environments (seas, lakes, and rivers) directly through activities like swimming and sunbathing, and indirectly via wastewater (Carve et al., 2021; Mokh et al., 2022). Although some studies found that concentration of UV filters (specifically for OCR and BP-3) in seawater might be higher than safe levels for marine life (Cadena-Aizaga et al., 2022), the typical levels fall below $1 \mu\text{g L}^{-1}$.

Detecting organic UV filters is challenging due to their low concentrations (in the ng L^{-1} to $\mu\text{g L}^{-1}$ range) and the interference from the sample matrix (Oubahmane et al., 2023). To ensure the reliability and accuracy of results, appropriate sample preparation methods are essential to concentrate the target analytes (Hejji, 2023 #733). The sorbent-based extraction method SPE is the most widely preferred method, as it simultaneously facilitates trace analytes pre-concentration and interference removal in a single step (Montesdeoca-Esponda et al., 2012; Pedrouzo et al., 2011; Wang et al., 2018).

Rimini and Riccione, located along Italy's Adriatic coast, are renowned tourist destinations known for their sandy beaches and vibrant summer tourism. Thus, it is expected that during peak summer months, the increased use of personal care products, particularly sunscreens, leads to a higher influx of UV filters into the sea. However, to the best of our knowledge data on the occurrence of UV filters in seawaters of Adriatic Sea is not available in the current literature apart Venice lagoon for EHMC (Pizzini et al., 2024). Concentration data on sediments highlighted the importance of river as the main contributor of many contaminants in the Adriatic basin, but the touristic coastal areas were identified as a direct input of UV filters from recreational activities (Combi et al., 2016).

The aim of the present study was to propose an analytical methodology and to determine the presence of twelve common UV filters and one transformation product Z-EHMC in the dissolved fraction of coastal waters from six beaches of Rimini and Riccione in the summer season (2024). In fact, while many studies have reported on the presence of EHMC in the marine environment biota (Bordalo et al., 2025), information for the levels of the *cis* isomer is lacking. This study is crucial for advancing environmental risk assessment methodologies by providing essential data on the concentrations of UV filters

and their transformation products in coastal waters, thereby informing regulatory frameworks and mitigating potential ecological risk.

1.2. Materials and Methods

1.2.1. Standard and Reagents

The UV filters analysed in this study included (*E*)-2-ethylhexyl methoxycinnamate (E-EHMC; 98.5%, Dr Ehrenstorfer Augsburg, Germany), 4-methylbenzylidene camphor (4MBC; 99.8%, Alfa Aesar, Karlsruhe, Germany), benzophenone-4 (BP4; 98.7%), benzyl salicylate (BS; >99%), and menthyl anthranilate (MA; 99.9%, Fluka, St. Louis, MO, USA). ethylhexyl dimethyl PABA (EHPABA; 98%), octocrylene (OCR; 99.1%), homosalate (HMS; 99.9%), and ethylhexyl salicylate (EHS; >99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Benzophenone-3 (BP3; 99.9%), isoamyl methoxycinnamate (IAMC; 96%), and etocrylene (Eto; 99.7%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). All solvents and reagents were of analytical grade. Ethyl acetate (EtAc, 99.5 %), methanol (MeOH, 99.9 %), ultrapure water and acetonitrile (ACN, 99.9 %) were provided by Scharlab (Barcelona, Spain) and dichloromethane (DCM, $\geq 99.9\%$) was provided by Avantor (VWR, Randor, PA, USA). Stock solutions of all the compounds were prepared in MeOH at concentration of 1000 mg mL^{-1} and further dilutions were made from the stock. Stock and working solutions were stored in glass vials protected from light and kept in a freezer at -20°C . HLB and C18 CHROMABOND[®] SPE cartridges (500 mg, 6 mL) were provided by Machery-Nagel (Düren, Germany).

1.2.2. Sample collection

Seawater samples were collected from three different beaches in Rimini (Bathing zone 17, 18 and 20) and Riccione (Bathing zones 34, 87, and 110) at different locations, along the Italy's Adriatic coast (Figure 1). Sampling was carried out from July- September 2024, mostly around the noon. These months were selected due to peak recreational activities during summer.

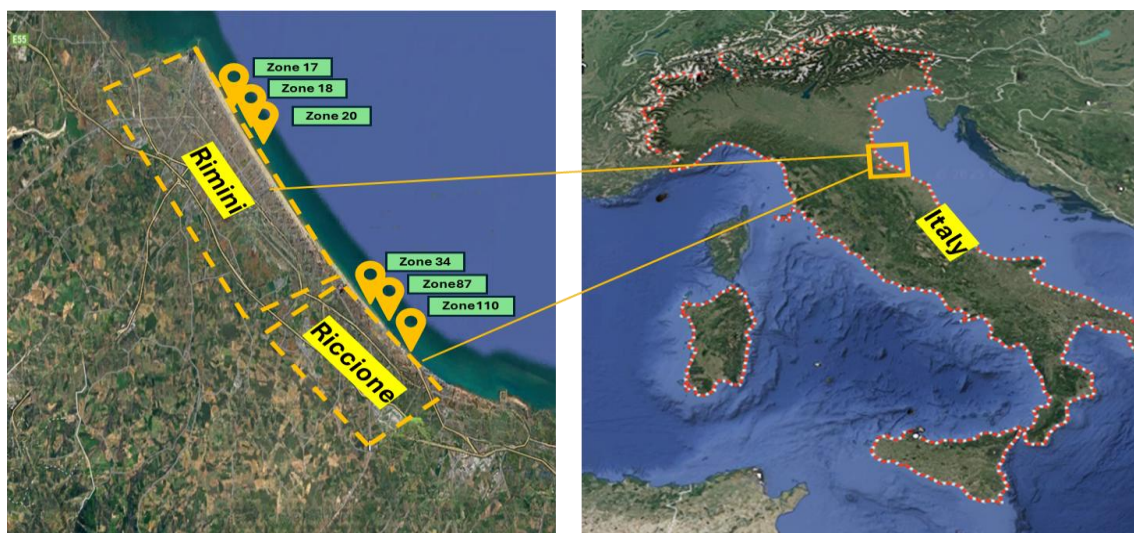


Figure 1. Sampling area and sites on the six beaches along the Adriatic Sea.

A total of 14 seawater samples were collected between 3-4 m from the shoreline, within the central area of the bathing zone, and approximately 50 cm below the water surface. All samples were stored in dark at 4 °C until analysis. Additional sample information is given in Table 1.

Table 1: Sampling details including sample names, collection date, time, area and location

Sample	Collection date	Collection Time	Collection Area	Location
Sa1	28/07/2024	1:00 -1:30 pm	zone 20	Rimini
Sa2	28/07/2024	1:00 -1:30 pm	zone 20	Rimini
Sa3	28/07/2024	1:00 -1:30 pm	zone 20	Rimini
Sa4	28/07/2024	1:00 -1:30 pm	zone 20	Rimini
Sb5	07/07/2024	02:30 pm	zone 17	Rimini
Sb6	07/07/2024	02:30 pm	zone 17	Rimini
Sc7	07/07/2024	02:00 pm	zone 18	Rimini
Sc8	07/07/2024	02:00 pm	zone 18	Rimini
Sd9	08/08/2024	12:30 pm	zone 110	Riccione
Sd10	08/08/2024	12:30 pm	zone 110	Riccione
S11	25/07/2024	03:30 pm	Zone 34	Riccione
S12	01/09/2024	05:00 pm	Zone 34	Riccione
S13	09/08/2024	11:00 am	Zone 87	Riccione
S14	13/08/2024	11:00 am	Zone 34	Riccione

In sample name, the same lower-case letter indicates similar sampling location and time.

Cosmetic samples labelled as containing EHMC (lip balm, hair oil and sunscreen) were purchased from local markets. (See Table 2).

Table 2. Commercial cosmetic products containing EHMC, including their manufacturer, product name, and listed composition.			
Cosmetic product	Company	Product Market Name	Composition
Hair Oil	Valquer Laboratorios S.L.U.	Aceite de Argán Capilar (Hair Oil)	Cyclopentasiloxane, Dimethicone, Phenyl Trimethicone, Isopropyl Palmitate, Parfum, Amodimethicone, Ethylhexyl Methoxycinnamate , Prunus Amygdalus Dulcis Oil, Argania Spinosa Kernel Oil, Rosa Moschata Seed Oil, Benzophenone-3, BHT, Benzyl Benzoate, Tocopheryl Acetate, Linalool, CI 47000, CI 26100
Lip Balm	Laboratorios Brum S.A.	Protector Labial SPF 25 Frambuesa (Lip Balm)	Microcrystalline Wax, Octyldodecanol, Ricinus Communis Seed Oil, Polydecene, Ethylhexyl Methoxycinnamate , Butyl Methoxydibenzoylmethane, Cera Alba, Octocrylene, Myristyl Myristate, Cetyl Palmitate, Butyl Methoxydibenzoylmethane, Cetyl Alcohol, Butyrospermum Parkii Butter, Aloe Barbadensis Leaf Extract, 12-Hexanediol, Caprylyl Glycol, Panthenol, Tocopheryl Acetate, BHT, Parfum, Linalool, Disodium EDTA.
Sunscreen	Laboratorios Kosei	Sun Facial Cream SPF 50+ Anti-Age (Sunscreen)	Aqua, Ethylhexyl Methoxycinnamate , Cyclopentasiloxane, Dimethicone, Ethylhexyl Salicylate, Polymethyl Methacrylate, Butyl Ethylbenzoylmethane, Glycerin, Potassium Cetyl Phosphate, Silica, Titanium Dioxide, Cetyl Alcohol, Octocrylene, PPG-15 Stearyl Ether, Vitis Vinifera Fruit Meristem Cell Culture, Squalane, Bis-PEG-18 Methyl Ether Dimethyl Silane, Glyceryl Stearate, Hydrogenated Vegetable Glycerides Citrate, Beta-Sitosterol, Polyglyceryl-6 Dioleate, PEG-100 Stearate, Propylene Glycol, Tocopheryl Acetate, Ascorbyl Palmitate, Lecithin, Xanthan Gum, Sodium Hydroxide, Citric Acid, Sodium Benzoate, Potassium Sorbate, Parfum, Caprylyl Glycol, Levulinic Acid, Sodium Levulinate, Hexyl Cinnamal, Linalool, Citronellol, Geraniol, Coumarin, Alpha-Isomethyl Ionone.

1.2.3. Photoisomerisation experiments

Due to the absence of a commercially available *cis* isomer of EHMC standard, *E*-EHMC was used for the quantification of *Z*-EHMC. This approach was already adopted in the literature (Poiger et al., 2004), and was supported by the observation that both isomers shared identical mass spectrometric fragmentation patterns and transitions during GC-

MS/MS analysis. This has been proved by laboratory experiments conducted to obtain Z-EHMC from *trans* isomer (*E*-EHMC). Aliquots of *E*-EHMC standard solution in ethyl acetate were placed in quartz cuvettes and irradiated in a photoreactor equipped with two UVC lamps ($\lambda = 254$ nm, 8 W, and 11 W; Philip, Holland) at a distance 9 cm, for 10 minutes. After irradiation, samples were analysed by GC-MS to identify and confirm the retention times and mass spectral and transitions of the *trans*- and *cis*- EHMC isomers (see Figure. 2).

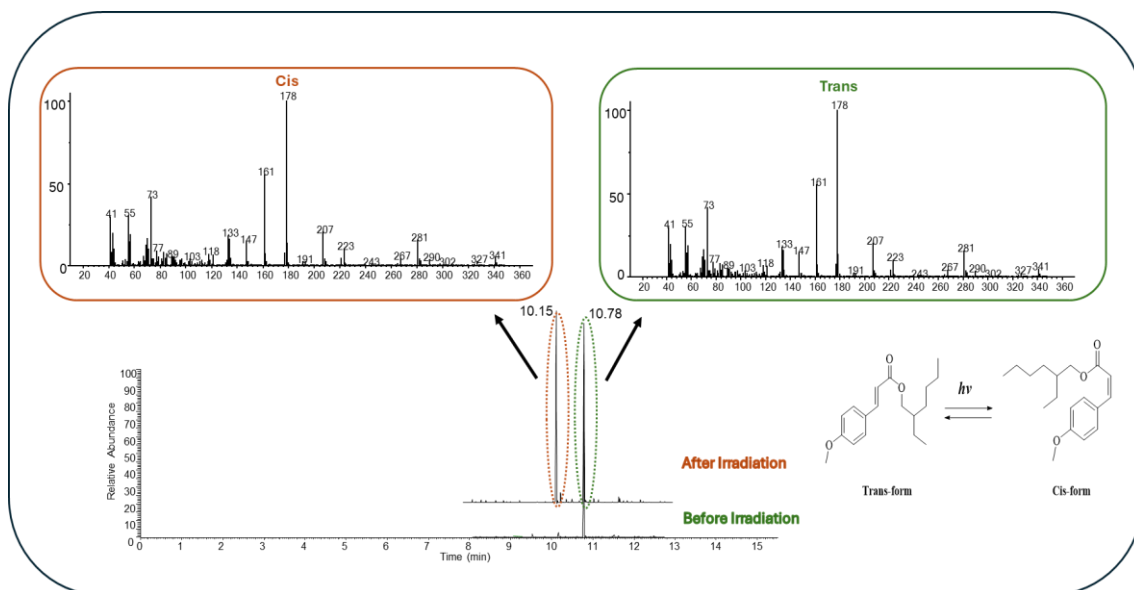


Figure. 2: Photoinduced isomerisation of *E*-EHMC (*trans*) to *Z*-EHMC (*cis*) after 10 min of UV lamp irradiation.

1.2.4. Extraction Procedure

The SPE extraction procedure was performed by modifying a method previously reported (Liu et al., 2011). A SPE method coupled with HPLC-DAD was initially optimised for two of the UV filters, *E*-EHMC and OCR. Later, under the optimised conditions (see section 3.2 discussion) the method was validated and applied to the determination of 12 UV and one transformation product using GC-MS/MS as detection and quantification technique. HLB and C18, SPE cartridge was used for extraction and each cartridge was preconditioned with 6 mL of DCM, 6 mL of MeOH followed by 6 mL of milli-Q water. The seawater samples ~400 mL were loaded onto the cartridges at a flow rate of 5 mL min⁻¹. A cleaning step (salt or impurity elimination) was carried out with 10 mL of Milli-Q water (5 % MeOH). Elution was done with 2 mL of MeOH (fraction discarded), and 4 mL of DCM. DCM extract was reduced to the final volume of 1 mL under the flow of N₂ gas, using an evaporation system.

1.2.5. Instrumental analysis

1.2.5.1. HPLC-DAD analysis

Chromatographic analyses were carried out using an Agilent Technologies SL 1200 Series HPLC system (Waldbronn, Germany) equipped with a binary pump and micro vacuum degasser. Separation was achieved on a ZORBAX SB-C18 column (150 × 4.6 mm, 3.5 µm). The gradient elution was programmed at a flow rate 2 mL min⁻¹: from 55% to 45% H₂O in 1 minute, 45-30% H₂O in next 10 minutes and then to 100% ACN in next 10 minutes. The injection volume was 20 µL and the column temperature was set at 40 °C. Detection was performed with UV/DAD detector set at 310 nm as the primary wavelength. Data acquisition and processing were managed using Agilent ChemStation software. Optimal sensitivity and peak resolution for two representative UV filters (OCR and E-HMC) were obtained using eluent A (ACN) and eluent B (H₂O). A standard solution containing both compounds was analysed, and the resulting chromatograms is shown in Figure. 3.

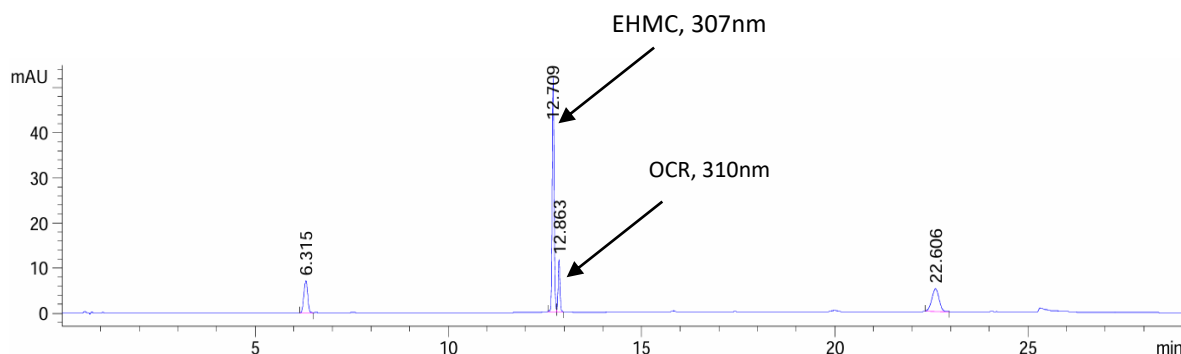


Figure. 3: HPLC chromatogram for the EHMC and OCR standards.

1.2.5.2. GC-MS/MS analysis

Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) was performed using a Thermo Scientific Trace 1310 GC system combined with a triple quadrupole mass spectrometer (TSQ 8000) and an IL 1310 autosampler (Thermo Scientific San Jose, CA, USA).

The chromatographic GC-MS/MS conditions for the determination of the target UV filters were previously developed by the authors (Vila et al., 2017; Vila et al., 2015). Separation was achieved on a Zebron ZB-semiVolatiles capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Phenomenex). Helium (purity 99.999%) was used as the carrier gas at constant flow rate of 1.0 mL min⁻¹. The oven program was set as follows: initial temperature 100 °C (held 1 min), ramped to 290 °C at 25 °C min⁻¹ (held 10 min),

and then increased to 320 °C (final hold, total run time $\approx$ 18.5 min). Samples were introduced in surged splitless mode (200 kPa, 1.20 min), with the injector maintained at 260 °C. The MS was operated in electron impact (EI) positive ionisation mode at +70 eV, with transfer line and ion source temperatures held at 290 °C and 350 °C, respectively. MS/MS acquisition was optimised using the auto-selected reaction monitoring (SRM) tool in the TSq 8000, selecting two to three transitions per compound to improve selectivity. The SRM transitions and corresponding collision energies are summarised in Table 3. Instrument control and data processing were carried out with Xcalibur 3.0 and Trace FinderTM 3.2 software.

Table 3 GC-MS/MS: retention times and selected SRM transitions.				
Acronym	INCI Name^a	RT (min)	SRM (m/z)^b	CE (eV)^c
EHS	Ethylhexyl salicylate	8.64	120 $\rightarrow$ 92	10
			138 $\rightarrow$ 120	10
			250.1 $\rightarrow$ 120	15
BS	Benzyl salicylate	9.00	91 $\rightarrow$ 65	15
			228.1 $\rightarrow$ 91	10
HMS	Homosalate	9.04	120 $\rightarrow$ 92	10
			138 $\rightarrow$ 120	10
			262.2 $\rightarrow$ 120	15
IAMC	Isoamyl methoxycinnamate	9.61	161 $\rightarrow$ 133	10
			178.1 $\rightarrow$ 161.1	10
			248.1 $\rightarrow$ 178	10
BP3	Benzophenone-3	9.68	151 $\rightarrow$ 95	10
			227.1 $\rightarrow$ 127.9	35
			227.1 $\rightarrow$ 184	20
BP4	Benzophenone-4	9.68	151 $\rightarrow$ 95	10
			227.1 $\rightarrow$ 127.9	35
			227.1 $\rightarrow$ 184	10
4MBC	4-Methylbenzylidene camphor	9.79	127.9 $\rightarrow$ 102	20
			170.6 $\rightarrow$ 128.1	15
			254.1 $\rightarrow$ 239.2	10
MA	Menthyl anthranilate	9.95	119 $\rightarrow$ 91.8	10
			137 $\rightarrow$ 119	10
			275.2 $\rightarrow$ 137	10
Z-EHMC	<i>Cis</i> -Ethylhexyl methoxycinnamate	10.09	161 $\rightarrow$ 133.1	10
			177.9 $\rightarrow$ 133.1	20
			290.2 $\rightarrow$ 178.1	10
Eto	Etocrylene	10.12	231.9 $\rightarrow$ 176.5	20
			248 $\rightarrow$ 164.9	25
			276.9 $\rightarrow$ 248.1	10
EHPABA	Ethylhexyl dimethyl PABA	10.51	148 $\rightarrow$ 104.2	25
			165.1 $\rightarrow$ 148.6	25
			277.2 $\rightarrow$ 164.9	10
E-EHMC	<i>Trans</i> -2-Ethylhexyl methoxycinnamate	10.68	161 $\rightarrow$ 133.1	10
			177.9 $\rightarrow$ 133.1	20

			290.2 → 178.1	10
OCR	Octocrylene	12.27	232 → 203	20
			248 → 165	30
			360.2 → 276.1	20
^a International nomenclature of cosmetic ingredients. ^b Italicised values show the quantification transitions. ^c CE: collision energy (eV).				

The most intense transition was selected for quantification, while second and third transitions were used for identification and confirmation. Since BP3 and BP4 co-eluted and exhibited identical transitions, they were analysed and quantified together as a combined mixture. Z-EHMC and E-EHMC eluted at different time but showed the same transitions. Quantification of the target compounds was performed using the standard solutions of the compounds in DCM, since the extraction was quantitative and no matrix effect was observed.

1.3. Results and discussion

1.3.1. SPE optimization

The extraction and elution parameters that potentially affect the SPE process were optimised to achieve the best extraction efficiencies for the target UV filters using synthetic seawater (3.5% NaCl). Initially, the SPE process was optimised for two of target UV filters EHMC and OCR, since at the beginning we only had a LC-DAD instrument (see section 2.5.1). Later one the optimal conditions were tried with the other target compounds demonstrating suitability. For SPE optimization, different parameters were studied: cartridge type, sample pH, salting out effect, and elution solvent.

SPE extraction was carried out using HLB and C18 cartridges, employing identical conditioning and elution protocols. The sample (50 mL) was load through the cartridge and afterwards elution was carried out with a volume of 6 mL (2 mL MeOH and 4 mL DCM). The water sample was fortified at 100 µg L⁻¹ with UV filters. As depicted in Figure. 4a, HLB cartridges provided the best extraction efficiency, offering a significantly improved chromatographic response compared to C18 cartridges. This performance is consistent with literature reports demonstrating the broad retention capabilities of HLB sorbents for compounds with mixed hydrophobic and polar characteristics (Liu et al., 2011).

The influence of pH on analyte extraction was assessed at pH 3 (0.2 M HCl) and neutral seawater pH (~ 8). As can be seen in the Figure. 4b chromatographic responses at pH 3 were comparable to those at neutral pH. Since the analytes are non-ionisable under this

pH range hydrophobic interactions should enhance; on the other hand, acid addition risks co-exiting humic substances that can foul cartridges (Tayeh et al., 2025). Therefore, maintaining the native seawater pH simplifies sample preparation, avoiding hazardous acid handling, and it aligns with greener analytical practices without compromising extraction efficiency.

To probe the salting out-effect NaCl was added to seawater at 10% and 20% (w/v). A slight decrease in peak areas was observed compared to natural salinity (~3.5% NaCl), but overall recoveries remained similar (Figure. 4c). Since seawater already contains sufficient salt to induce portioning effects, further “salting-out” was unnecessary, reducing reagent consumption and streamlines the workflow.

Elution solvent also can have significant influence on the recoveries of the target compounds. The elution solvents first selected were taken from a previous study (Liu et al., 2011; Vila et al., 2017). For the optimization 3 different elution conditions and solvents were tested: 1) MeOH (2 mL, first fraction), DCM/MeOH (50:50; v/v) (4 mL, second fraction); 2) MeOH (2 mL first fraction), isopropanol (4 mL, second fraction), DCM (4 mL, third fraction); 3) MeOH (2 mL, first fraction), DCM (4 mL, second fraction). For all the experiments the MeOH extract (first fraction) did not show any peaks resulting inefficient for the extraction of the target analytes. Regarding the second fraction, DCM/MeOH gave low recoveries (between 10 and 30%), as well as isopropanol with low values. Only DCM gave satisfactory performance. Hence, the last conditions (case 3) were the ones selected analysing the DCM extract. The first solvent fraction by MeOH was kept as clean-up solvent and it was discarded, and the DCM fraction was analyzed.

Therefore, the optimised SPE method for UV filters in water samples is described as follows: raw water sample application without pH adjustment and salt addition, employing HLB cartridge, and eluting the target compounds with DCM (4 mL) after a cleaning step with MeOH (2 mL). This optimised method was further applied for the recovery study and method validation for the targeted compounds.

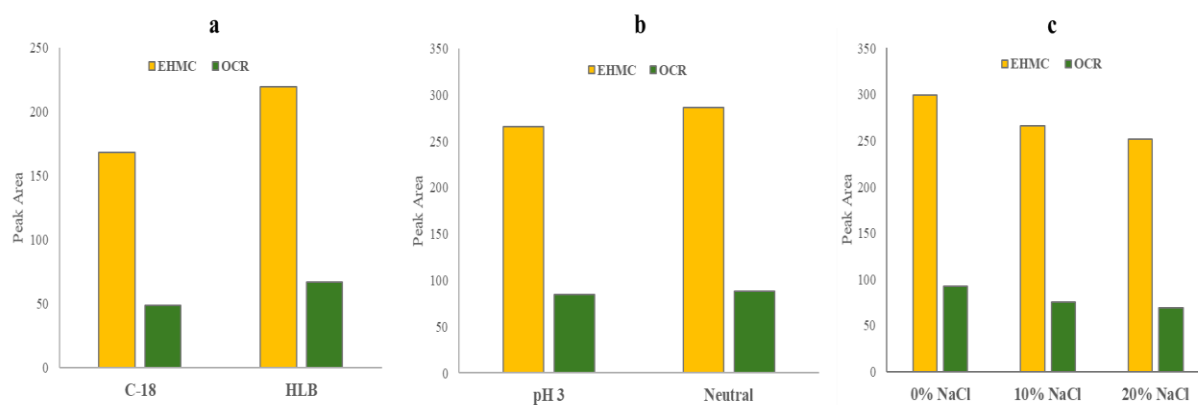


Figure. 4: Effect of (a) cartridge type, (b) sample pH (c) salting-out effect on the extraction of UV filters, EHMC and OCR.

1.3.2. Method Validation

To confirm method suitability for the quantitative determination of the 12 target UV filters in water, method performance parameters were assessed and are summarised in Table 2. The chromatographic run time was only 15 min. Analyses were carried out by GC-MS/MS (see conditions in section 2). The method was evaluated in terms of linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and recoveries. Calibration standards were prepared in DCM at twelve levels, covering a wide concentration from $0.1 \mu\text{g L}^{-1}$ to $500 \mu\text{g L}^{-1}$ (see Table 4 for compound specific ranges). Linear relationships between analyte concentration and chromatographic response with determination coefficients $R^2 \geq 0.994$ were confirmed. Instrumental precision, expressed as relative standard deviation (%RSD), was satisfactory, with intraday (n=3) and interday (n=5) values ranging from 1.8-7.0 % and 2.1-12 %, respectively.

Table 4. Performance of the SPE-GC-MS/MS method for the target compounds.							
Compounds	Linearity	R ²	Precision (RSD, %)		Recoveries (% ± RSD)		LOQ
	µg L ⁻¹		Intra-day n=3	Inter-day n=5	Seawater	Spring water	ng L ⁻¹
EHS	0.2-500	0.9982	2.3	5.5	77.3 ± 5.5	107.0 ± 4.7	0.73
BS	1-500	0.9989	11	12	87.3 ± 7.3	91 ± 14	0.40
HMS	1-500	0.9993	2.0	2.5	74.4 ± 2.2	69 ± 13	0.49
IAMC	1-500	0.9996	1.8	3.2	98.0 ± 6.3	93 ± 11	0.30
BP 3-4	5-500	0.9942	2.4	11	74.4 ± 6.3	96.0 ± 6.3	3.4
4MBC	5-500	0.994	6.5	9.3	87.4 ± 3.3	102 ± 11	1.9
MA	0.1-500	0.999	1.6	5.9	90.1 ± 2.9	94.1 ± 6.0	0.17
Eto	0.2-500	0.9998	1.6	4.8	107 ± 12	88 ± 11	0.10
EHPABA	0.1-500	0.9997	4.5	12	80 ± 13	78.4 ± 5.4	0.14
E-EHMC	1-500	0.9996	4.7	2.1	95.3 ± 8.8	72.4 ± 8.8	0.94
OCR	1-500	0.9994	7.0	8.0	104 ± 12	89 ± 12	0.56
							1.9

Recovery studies were performed at two concentration levels using real water samples (400 mL): spring water and seawater spiked at $0.02 \mu\text{g L}^{-1}$ and $0.1 \mu\text{g L}^{-1}$, respectively. Recoveries were determined as the ratio of the response obtained from spiked samples to that of standards at the corresponding concentration level and expressed as percentages. For the seawater matrix, a sample collected during the winter season was selected to minimize the likelihood of the background contamination of the UV filters in procedural blanks confirming the absence of the target compounds, ensuring accurate recovery assessment. Figure. 5, shows satisfactory recovery values for both seawater and spring water, generally higher than 80 % with the $\pm\text{SD}$ values between 2 and 14 %. The LODs and LOQs were calculated as the concentration in real sample giving a signal-to-noise ratio of three ($S/N = 3$) and ten ($S/N = 10$), respectively for each compound. The method demonstrated high sensitivity with LODs below 1 ng L^{-1} for most compounds (see Table 2).

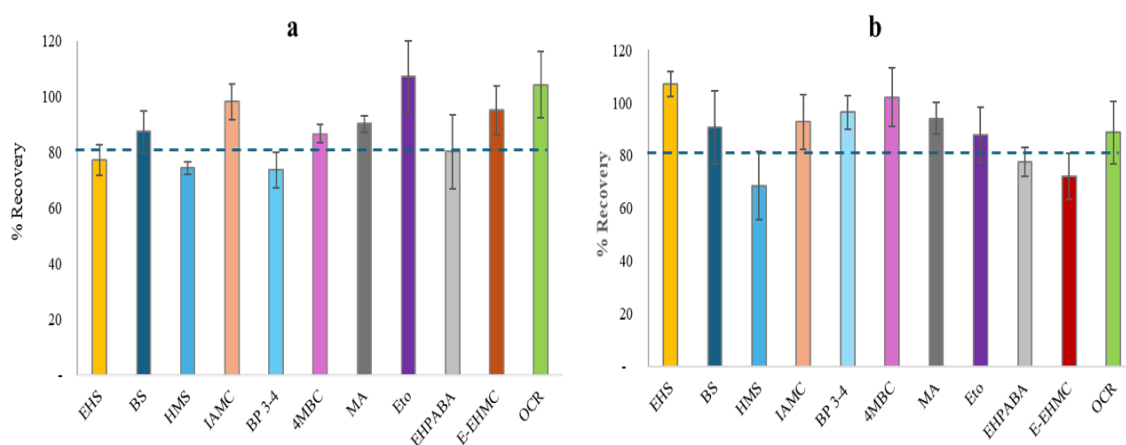


Figure. 5: Recoveries obtained for (a) seawater, and (b) spring water.

1.3.3. Application to seawater samples

The validated SPE-GC/MS method was applied to 14 seawater samples collected during the recreational activities from six beaches along the Adriatic coast. All 12 target UV filters and the transformation product Z-EHMC were detected across the sampling sites, confirming their ubiquitous presence in coastal waters. Figure. 6 displays SRM reconstructed SPE-GC–MS/MS chromatograms of a representative seawater sample (S4) containing all the analytes.

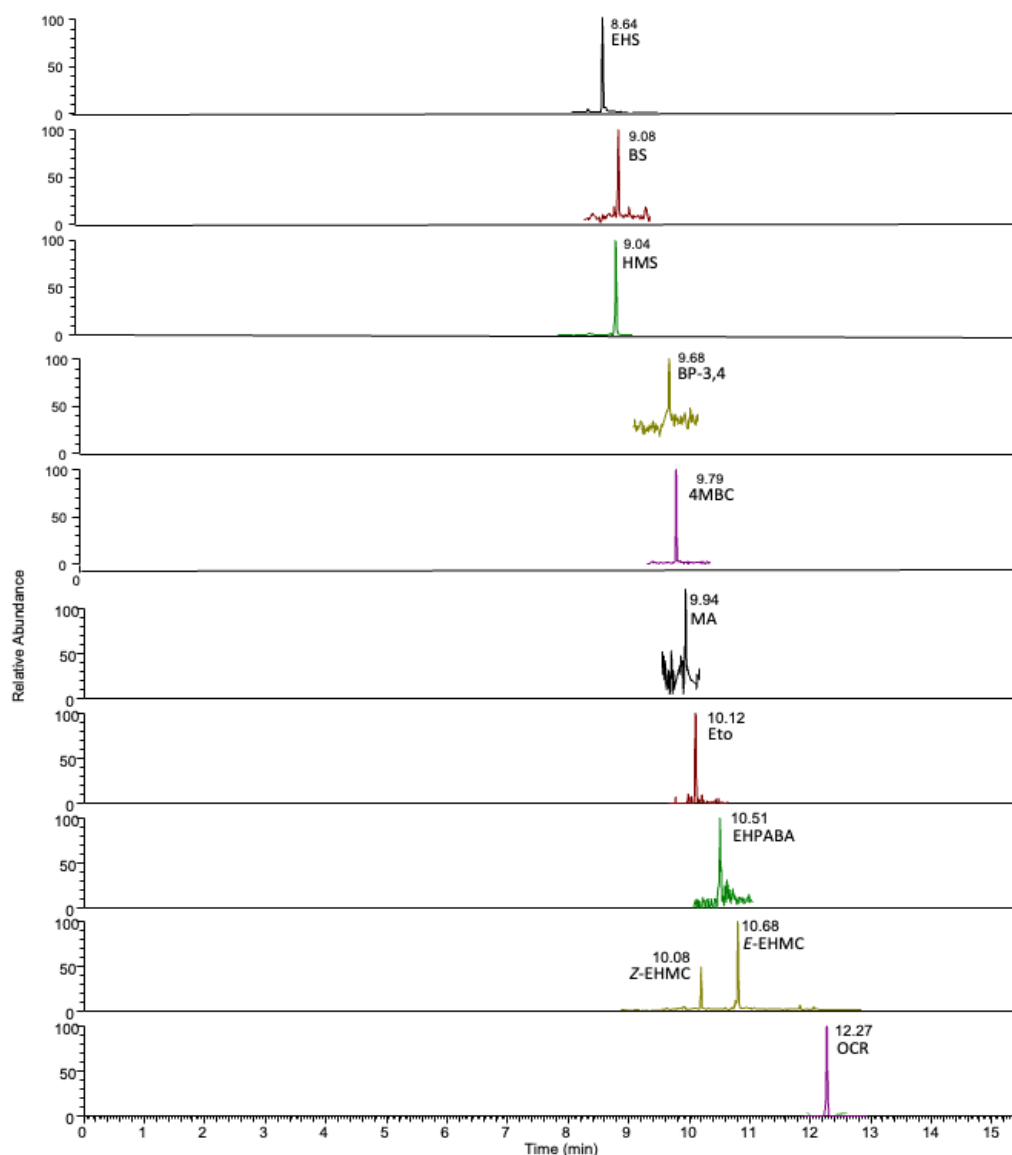


Figure. 6. SRM reconstructed SPE-GC-MS/MS chromatograms of a real seawater sample (Sa4).

Table 5 summarizes the quantification results for each analyte and the summed concentrations of UV filters. Compounds consistently detected in all samples were EHS, BS, HMS, BP-3,4, Z-EHMC, *E*-EHMC and OCR. Eto and IAMC were detected in seven and six of the fourteen samples, respectively. This trend reflects the widespread use of the UV filters in personal care products. On the other hand, Eto and MA which are prohibited in EU cosmetics formulations [3], were detected in few samples at low levels ($< 3 \text{ ng L}^{-1}$). In fact, MA appeared only once (Sa4).

Total UV filter concentrations of all detected UV filters spanned from 0.2 to 10 $\mu\text{g L}^{-1}$, with the exception of S11 (Table 3). Sample S11 exhibited the highest overall UV filter load (53 $\mu\text{g L}^{-1}$), with extreme levels of OCR (23,100 ng L^{-1}), HMS (16,400 ng L^{-1}), and EHS (10,900 ng L^{-1}). These extreme values could represent an anecdotal scenario due to concomitant factors such as a relatively high number of bathers (10 people were counted nearby sampling). Samples Sd9 and Sd10 also showed elevated levels of several UV filters, including OCR (7,317 ng L^{-1} and 6,830 ng L^{-1} , respectively) in agreement with other studies on locations nearby important cities (Tsui et al., 2014). In the other 11 samples, the concentrations of all the investigated UV filters fall well below 10^3 ng L^{-1} concordantly with literature (Grant et al., 2025). Replicate sampling showed a rather high variability for each single analyte, with relative standard deviation (RSD) lowest for OCR and highest for *E*-EHMC.

Table 5. Concentrations (ng L⁻¹) of single UV filters and total concentrations (µg L⁻¹) in seawater samples.

Sample name	EHS	BS	HMS	IAMC	BP 3-4	4MBC	MA	Z-EHMC	Eto	EHPABA	E-EHMC	OCR	Total
Sa1	159	4.8	150		90	51		47	1.6	0.55	219	727	1.4
Sa2	224	10	187		62	90		50	2.0	0.49	112	556	1.3
Sa3	223	10	204		62	122		43	2.6	0.29	45	661	1.3
Sa4	78	5.4	133		66	97	<LOQ	20	2.2	0.37	43	712	1.1
Sb5	31	6.1	26		52	12		7.5	0.4		64	101	0.28
Sb6	20	4.0	17		120			4.8	0	0.26	18	96	0.22
Sc7	27	5.6	24		154	54		5.7			19	126	0.34
Sc8	19	4.8	17		172	37		3.7			13	123	0.30
Sd9	319	6.7	2020	4.8	60	26		195	1.2	4.1	157	7317	10
Sd10	228	9.3	1560	2.5	50	25		149		3.2	84	6830	8.9
S11	10900	16	16400	223	44			1008		5.4	1474	23100	53
S12	206	8.2	163	29	44	17		73	1.3	3.8	74	406	1.0
S13	103	7.1	81	15	54			52			224	491	0.99
S14	16	3.0	18	2.5	66			21			39	74	0.21

*In sample name, the same lower-case letter indicates similar sampling location and time.

**Empty cells indicate UV filters not detected

Values in italics are estimated, below LOQ.

The UV filter *E*-EHMC was found in all the analysed samples at significant levels (1-19 % of the total mass of target UV filters) and was the second most abundant UV filter in the samples. Notably, its *cis* isomer (*Z*-EHMC) occurred at similar if not higher (samples Sd9 and Sd10) concentrations in comparison to the *trans* form (Figure. 7). The comparison with other water systems was challenging as the *cis* isomer was rarely analysed in environmental samples (Liu et al., 2011), or it is analyzed as a sum of isomers (Langford & Thomas, 2008; Tashiro & Kameda, 2013). Plagellat et al. analysed both *E*- and *Z*-EHMC in sewage sludge from different wastewater treatment plants in Switzerland, but the concentrations of the single isomers were not reported (Plagellat et al., 2006).

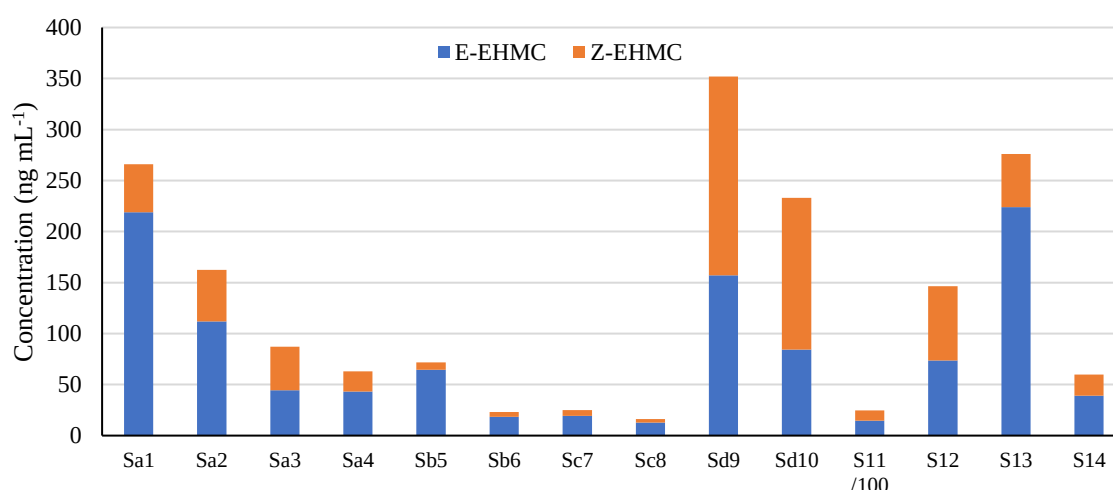


Figure. 7: Comparative concentration of *E*-EHMC and *Z*-EHMC in the fourteen real Adriatic seawater samples.

OCR exhibited the highest concentrations in all the investigated samples (74-23,100 ng L⁻¹) is coherent with the study by Combi et al. found, that OCR was the most abundant UV filter in the sediments of Adriatic Sea with highest concentrations in the northern sector (16.3 ± 9.6 ng g⁻¹) (Combi et al., 2016). Among the other two UV filters investigated by the authors, EHMC was more abundant than BP-3, similarly to what observed in our samples where (*E*+*Z*)-EHMC > BP-3,4. The concentrations of *E*-EHMC in seawater from various locations exhibited large variability, generally in the < 1-100 ng L⁻¹ range in accordance with our data (13-224 ng L⁻¹) with the exception of sample 11. When considering the Adriatic basin, the concentrations of EHMC in the Venice lagoon were reported to fall in the 0.23-22.4 ng L⁻¹ range with the highest concentrations observed in summer and attributed to effluents from wastewater treatment plants (Pizzini et al., 2024). This range was considered among the lowest in natural waters worldwide

where the upper limit exceeded 10^3 ng L^{-1} (Pizzini et al., 2024) underlining the importance of direct introduction from recreational activities (e.g. swimming, bathing). Oxybenzones (BP-3,4) were detected at rather low concentrations $22\text{--}86 \text{ ng L}^{-1}$ in all the samples, on average similar to the levels observed in bathing areas of the Western Mediterranean Sea (Gandar et al., 2025) and lower than the levels reported in the tourist resorts in the Black Sea (Chiriac et al., 2021).

These findings supported the widespread presence and varied distribution of UV filters in coastal environments, likely influenced by intense seasonal recreational activities and coherent with the fact that in cosmetic formulations different UV filter are mixed to obtain a broad-spectrum coverage against the diverse UV radiation. The detection of Z-EHMC in notable concentration is of particular concern, emphasizing the ecological relevance of monitoring these compounds in marine ecosystems.

1.3.4. On the origin of the Z-EHMC in seawater samples

To determine whether the Z-EHMC detected in environmental seawater samples originates directly from cosmetic formulations during the manufacture or storage of the product or is instead formed through environmental transformation, a targeted analysis was conducted on commercial products. Cosmetic products explicitly labelled as containing EHMC were selected for this investigation. As mentioned, the isomer used on the cosmetic formulations is the *trans* (*E*-EHMC). The selected products included quite diverse formulations as hair oil, lip balm and sunscreen, widely used by the general population and in recreational coastal settings.

Given the typically high concentrations of EHMC in such formulations required to act as a UV-filter sometimes reaching the percentage, a small amount of sample (5-8 mg) were weighted and homogenised in 10 mL of solvent using vortex and ultrasound agitation. Then an aliquot was diluted and finally analysed by GC-MS. Importantly, in all cases, only the *E*-EHMC was detected, with no observable presence of the Z-EHMC, indicating that Z-EHMC is not inherently present in the original cosmetic formulations.

The *trans* to *cis* photoisomerisation of EHMC is a well-known process that can occur very rapidly under light exposure (Huong et al., 2007; Pattanaargson et al., 2004). In fact, as commented in section 2, a solution containing *E*-EHMC was irradiated with the intention of obtaining the *cis* isomer (Z-EHMC). After 10 min the solvent was analyzed

and both isomers were present in the solution at quite similar quantity (see Figure 2, showing the chromatogram and the two mass spectra).

The percentage of Z-EHMC to (E+Z)-EHMC in the investigated real seawater samples from the Adriatic Sea (see Table 3 and Figure 7) occurred in a range between 18-64 % (33 % on average) underpinning the importance of this isomer. At the best of our knowledge, the percentage of the *cis* isomer in samples of environmental interest was reported only for sewage sludge where on average accounted for 40 % of total EHMC (Plagellat et al., 2006).

These findings suggest that the Z-EHMC detected in seawater samples is not released from the original cosmetics, but it is formed probably due to environmental conditions upon exposure to solar radiation of E-EHMC. As photoinduced E-EHMC isomerisation occurs readily in different media (Huong et al., 2007), several sources of Z-EHMC in seawater can be considered: direct photoconversion of E-EHMC in situ in the water, wash-off from sun exposed skin after sunscreen application, and contributions from wastewater already containing the *cis* isomer. Elucidating these formation pathways is critical for accurately assessing the environmental impact of EHMC on seawater quality.

1.4. Conclusions

A single SPE based sample preparation method coupled with GC-MS/MS was developed and successfully applied for the analysis of 12 UV filters and the transformation product Z-EHMC in seawater samples. The method demonstrated excellent analytical performance with the detection limits in the ng L⁻¹ range and recovery efficiencies exceeding 80%, making it suitable for environmental monitoring of these emerging contaminants in marine ecosystems.

The widespread occurrence of UV filters was confirmed across 14 seawater samples from popular Adriatic beaches, with the target compounds detected at varying concentrations. These contaminants, underscoring their potential environmental impact, notably high concentrations of certain UV filters were observed, particularly EHS (10,900 ng L⁻¹), and OCR (23,100 ng L⁻¹), but most of the samples exhibited concentrations of UV filters lower than 1 µg L⁻¹. A critical finding was the ubiquitous presence of Z-EHMC in all samples (18–64 % of total E/Z-EHMC), despite its absence in commercial formulations, confirming significant in-situ photoisomerisation of the parent E-EHMC under natural

solar radiation. The environmental transformation process generates a potentially more toxic isomer that current regulatory frameworks fail to address.

These findings provide critical insight into the environmental fate of UV filters and highlight the need for further monitoring and risk assessment, especially considering the increasing use of these compounds in personal care products. This research also contributes to the growing body of knowledge regarding contaminants of emerging concern in aquatic ecosystems, calling for improved regulatory measures and more stringent monitoring to mitigate the potential ecological risks associated with UV filters.

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2. Photoisomerization of the UV filter Ethylhexyl Methoxycinnamate in Aquatic Environments, in cosmetics and in *in vitro* human skin: Environmental and health Implications and Persistence

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2.1. Introduction

2-Ethylhexyl 4-methoxycinnamate (EHMC), also known as octinoxate, is a widely used organic UV filter commonly incorporated in personal care products (PCPs), such as sun care products, facial treatment products, lipstick, hand and body, presenting a high absorption capacity in the UVB region (290–320 nm) (Gackowska et al., 2018; MacManus-Spencer et al., 2011; Manová et al., 2013). The maximum allowed concentration of EHMC in PCPs is 10% in Europe, according to Annex VI List of UV Filters Allowed in Cosmetic Products of the EU Cosmetic Regulation, and slightly smaller concentration 7.5% is valid in USA (European Commission, 2024; Janjua et al., 2008).

A critical limitation of EHMC as a photoprotective agent stems from its photochemical instability, leading to a decrease in UV absorption efficiency. Pattanaargson & Limphong, (2001), and other authors (see examples below) studied that EHMC undergoes degradation when exposed to light, but it remains stable when kept in the dark at various temperatures (up to 60°C). This photoisomerization process significantly affects the photoprotective efficacy of sunscreen cream products. The *E*-isomer exhibits a maximum absorption wavelength at approximately 310 nm with a high molar extinction coefficient of 19,500 L mol⁻¹ cm⁻¹, whereas the *Z*-isomer shows reduced UV absorption with a maximum at 312 nm and a substantially lower molar extinction coefficient of 10,000 L mol⁻¹ cm⁻¹ (da Silva et al., 2022). Studies have demonstrated that this isomerization can result in SPF reductions exceeding 30%, with the photoisomerization of EHMC potentially causing significant loss of UV filtering efficiency (Huong et al., 2007).

Previous investigations have primarily focused on photoisomerization kinetics under controlled laboratory conditions using simulated/natural light sources (MacManus-Spencer et al., 2011; Vione et al., 2015). This transformation has raised significant concerns regarding its safety and environmental impact, particularly since the *Z*-isomer has been shown to exhibit increased genotoxicity and potential endocrine disrupting effects compared to the parent *trans* isomer, raising concerns about its safety upon skin

exposure and environmental release (Nečasová et al., 2017). Endocrine disrupting potential of EHMC has been suggested in several *in vitro* and *in vivo* studies (Christen et al., 2011; Zucchi et al., 2011). Its lipophilic nature facilitates bioaccumulation in aquatic biota of different trophic levels (Fent, Kunz, et al., 2010; Fent, Zenker, et al., 2010). The effect of this compound on marine life and its potential for bioaccumulation require more detailed investigation, especially as it has been detected in water bodies worldwide, including the *cis* isomer although no quantitative data are available, highlighting the need for comprehensive studies on its environmental fate (Barón et al., 2013; Bordalo et al., 2025; Rodil et al., 2012; Santos et al., 2012; Tarazona et al., 2010; Zuloaga et al., 2012). Studies have shown the presence of EHMC along with other UV filters in human breast milk (Liu et al., 2022), urine (Huang et al., 2019), and even in marine animals (shellfish) in the Mediterranean and Atlantic coast of France (Bachelot et al., 2012). In light of recent SCCS evaluations flagging *E*-EHMC for its potential endocrine-disrupting effects (Bernauer et al., 2025), investigating both the parent compound and its transformation product is essential for comprehensive environmental risk assessment. However, these evaluations do not account for the broader environmental exposure risk or the long-term ecological effects or behaviour of EHMC photodegradation.

Despite the extensive body of research detecting EHMC in many environmental waters, while others characterizing EHMC photoisomerization, critical knowledge gaps persist. Previous studies have predominantly employed artificial light sources, standardized solvents, and concentrations far from real-world exposure scenarios. The present study addresses these fundamental gaps by investigating EHMC photoisomerization under environmentally and dermally relevant conditions. This work examines photoisomerization in real water samples (seawater, river water, and swimming pool water) at low concentrations (parts per billion). In addition, *E/Z* transformation is also monitored in authentic commercial cosmetic products containing much higher concentrations than those in the environmental waters (*E*-EHMC at percentage) under simulated UV and natural sunlight, and on an artificial human skin model (VITRO-SKIN®) (Alvarez-Rivera et al., 2016) to evaluate the EHMC degradation and formation of the *Z*-EHMC isomer during realistic exposure scenarios.

2.2. Experimental section

2.2.1. Reagents and material

(*E*)-2-Ethylhexyl methoxycinnamate (*E*-EHMC, 98.5%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Ethyl acetate (EtAc, 99.5 %), hexane (Hex, > 99%) and ultrapure water were provided by Scharlab (Barcelona, Spain). Methanol (MeOH, 99.9%) was purchased from Sigma-Aldrich. All solvents and reagents were of analytical grade. A stock solution of *E*-EHMC was prepared in MeOH at concentration of 1000 mg L⁻¹ and further dilutions were made either in MeOH (for spiking water samples) or in EtAc and Hex (for direct injections). Stock and working solutions were stored in glass vials protected from light and kept in a freezer at -20°C.

The manual SPME holder and the 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber were purchased from Supelco (Bellefonte, PA, USA). Low-pressure mercury lamp (11 W, $\lambda = 254$ nm) was provided by OSRAM (Munich, Germany). Artificial skin (VITRO-SKIN®) was acquired from IMS testing group (ME, USA). VITRO-SKIN® (IMS-USA) is an advanced testing material formulated to closely replicate the surface characteristics of human skin, including its topography, pH, critical surface tension, and ionic strength. Glass slides and quartz cuvettes were provided by Labbox (Barcelona, Spain).

Various real water samples were obtained from various locations in Galicia (NW Spain): seawater, river water, and swimming pool water. All these samples were collected (30 mL - 1 L) in glass vials or bottles, transported and stored at -20 °C in the dark.

Cosmetic products explicitly labelled as containing EHMC were selected for this investigation. The samples were acquired in local stores and included a sunscreen cream, a lip balm, and a hair oil. The lip balm and hair oil samples had been previously used by consumers, whereas the sunscreen cream was acquired for this study. All products had been purchased 1–2 years prior to the analysis.

2.2.2. Photodegradation in water

100 mL of water samples (ultrapure water, seawater, river water, and swimming pool water) were spiked with *E*-EHMC at a concentration of 20 µg L⁻¹. Aliquots of 2.5 mL were placed in quartz cuvettes and irradiated with UV light (11 W, $\lambda = 254$ nm) at distance of 4 cm for the designated time (0, 10, 20, 40, 60 min). Dark tests (in the absence of UV irradiation) were also performed by placing the cuvettes at the same distance, maintaining

the UV light lamp switched off for the correspondent time to assure that no isomerization occurred in the absence of light. Afterwards, the samples (non-irradiated and irradiated) were extracted using SPME and analyzed by GC-MS.

The SPME experimental procedure was performed by using a method previously reported with minor modifications (Vila et al., 2016). 2 mL of the sample were transferred into 10 mL glass vials sealed with aluminium caps furnished with PTFE-lined septa and placed in a water bath maintained at 100 °C, letting them equilibrate for 3 minutes. Subsequently, the PDMS/DVB was exposed to the vapor phase above the water sample (i.e., in headspace mode) for 10 minutes. After extraction, the fiber was inserted and immediately thermally desorbed in the GC injection port at 260 °C for 3 minutes, followed by GC–MS analysis. To prevent memory effects, blank samples were periodically performed using ultrapure water following the same SPME procedure.

2.2.3. Photodegradation of cosmetic products in different substrates: glass slides and artificial skin model

About 0.02 g of each cosmetic product (sunscreen cream, lip balm, and hair oil) were applied either to a glass slide (2.5 × 3.7 cm) or to a 3 × 3 cm piece of artificial skin (VITRO-SKIN®) (Alvarez-Rivera et al., 2016). The product was carefully spread over the surface using circular motions with a finger to form a thin, uniform film. The samples were then subjected to irradiation under two different light conditions: a UV light lamp (11 W, $\lambda = 254$ nm) in a home-made photoreactor (see Figure 1) and natural sunlight at the external laboratory terrace. The exposure times were 30 min to 4 hours. The same samples were also prepared and maintained protected from the light to perform the corresponding dark tests. Both substrates were placed on a watch glass. For the artificial skin, the cosmetic product was applied to the “skin topography” side, which was always facing the light.

To perform the analysis, irradiated and non-irradiated samples were placed in falcon tubes and 10 mL of ethyl acetate were added. Then the tubes were immersed in an ultrasound bath at room temperature and sonicated for 15 min (50 kHz, 25 °C approximately). Afterwards, the solvent extract was diluted before GC-MS analysis. The experiments were done per duplicate.

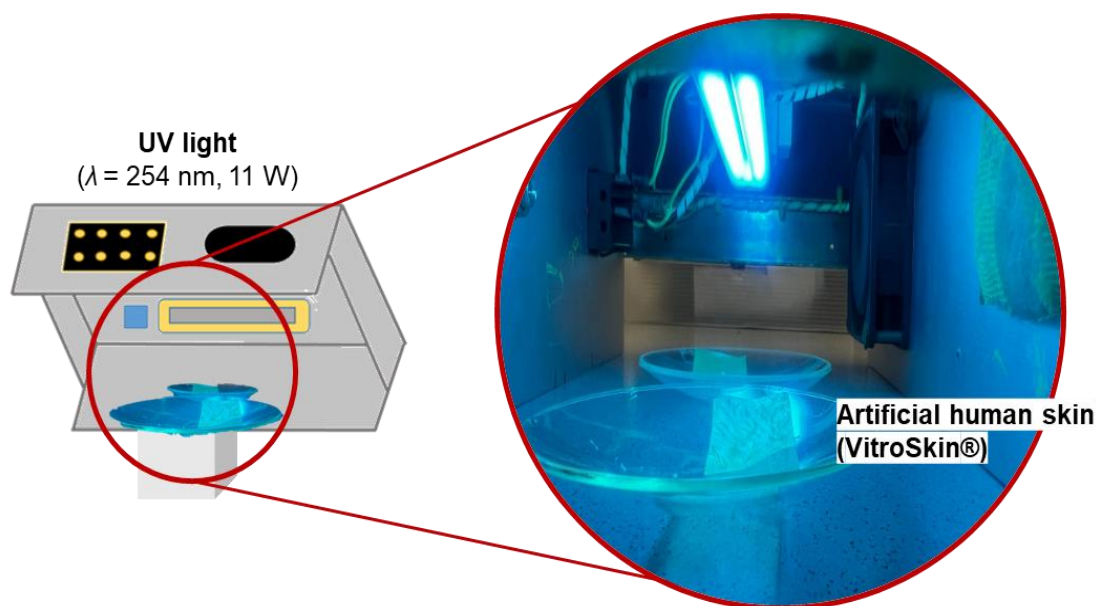


Figure 1. Representation of artificial skin exposure under UV light.

2.2.4. Gas chromatography-mass spectrometry analysis

Analyses were conducted on an Agilent 7890A gas chromatography coupled to an Agilent 5975C mass selective detector featuring a triple-axis detector. A 30 m $\times$ 0.25 mm $\times$ 0.25 μ m ZB-Semivolatiles column (Phenomenex, Torrance, CA, USA) was employed for chromatographic separation. High purity helium (99.999 %) served as the carrier gas at a flow rate of 1.0 mL min⁻¹. The oven program was set as follows: initial temperature 100 °C (held 1 min), ramped to 290 °C at 25 °C min⁻¹ (held 4 min), making a total run time less than 14 min. The transfer line, ion source, and quadrupole were maintained at temperatures of 290 °C, 150 °C, and 300 °C, respectively. The mass spectrometer was run in positive electron impact (EI) ionization mode at +70 eV. The injection temperature was 260 °C. The transfer line, ion source and quadrupole were maintained at temperatures of 290, 150, and 300 °C, respectively. Z-EHMC and E-EHMC were analysed in full scan (FS) mode. The quantification ion was m/z 178, while the identification ions were m/z 161 and m/z 133. The retention times for Z-EHMC and E-EHMC are 8.2 and 8.7, respectively. The instrument was controlled with Agilent MSD ChemStation software (version E.02.00.493).

In the case of the cosmetic samples, most analyses were performed using a Thermo Scientific Trace 1310 GC system combined with a triple quadrupole mass spectrometer (TSQ 8000) and an IL 1310 autosampler (Thermo Scientific San Jose, CA, USA). Separation was achieved on a Zebron ZB-Semivolatiles column (30 m $\times$ 0.25 mm i.d.,

0.25 μm film thickness, Phenomenex). Helium (purity 99.999%) was used as the carrier gas at constant flow rate of 1.0 mL min^{-1} . The oven program was set as follows: initial temperature $100\text{ }^{\circ}\text{C}$ (held 1 min), ramped to $290\text{ }^{\circ}\text{C}$ at $25\text{ }^{\circ}\text{C min}^{-1}$ (held 4 min), making a total run time less than 14 min. Samples were introduced in surged splitless mode (200 kPa, 1.20 min), with the injector maintained at $260\text{ }^{\circ}\text{C}$. The MS was operated in electron impact (EI) positive ionisation mode at $+70\text{ eV}$, with transfer line and ion source temperatures held at $290\text{ }^{\circ}\text{C}$ and $350\text{ }^{\circ}\text{C}$, respectively. The SRM transitions (m/z) and corresponding collision energies (eV) for both isomers were: $290.2 \rightarrow 178.1$ (10), $161 \rightarrow 133.1$ (10), and $177.9 \rightarrow 133.1$ (20) [26, 27]. The retention times for *Z*-EHMC and *E*-EHMC are 10.07 and 10.68 minutes, respectively. Instrument control and data processing were carried out with Xcalibur 3.0 and Trace FinderTM 3.2 software.

2.3. Result and Discussion

2.3.1. Photoisomerization of EHMC in various water matrices

Several water samples, including ultrapure water and real environmental waters such as seawater, river water, and swimming pool water, were spiked with *E*-EHMC to a final concentration of 20 ng mL^{-1} . Photodegradation experiments were conducted in a home-made photoreactor containing a UV light lamp (11 W, $\lambda = 254\text{ nm}$). Aliquots of 2 mL from these spiked samples were either analysed directly or after photodegradation under UV light, following the procedure described in Section 2. Both irradiated and non-irradiated samples were analysed by SPME-GC-MS. The irradiated aliquots were exposed for different periods of time (0, 10, 20, 40, and 60 min) before analysis and the results are presented in Figure 2. Figure 3 shows the SPME-GC-MS ion chromatogram (ion m/z 178) of seawater and swimming pool water samples obtained for the different times.

The analysis of the non-irradiated samples (t_0) shows the single presence of the *E*-EHMC whereas the *cis* isomer (*Z*-EHMC) was not found (see Figures 1 and 2). However, all the other samples showed the presence of both isomers confirming the EHMC photoisomerization (*E/Z*). In all the irradiated samples, the *Z* isomer was predominant (irradiation time 10, 20, 40, 60 min). The chromatographic response for each one of the isomers was quite similar independently the exposure time showing such *E/Z* stationary state. Other authors have observed it in organic and aqueous media (Herzog et al., 2019; Jentzsch et al., 2016; Jentzsch et al., 2019; MacManus-Spencer et al., 2011; Morliere et

al., 1982), in which the total amount of EHMC appears quite similar indicating a poor EHMC elimination.

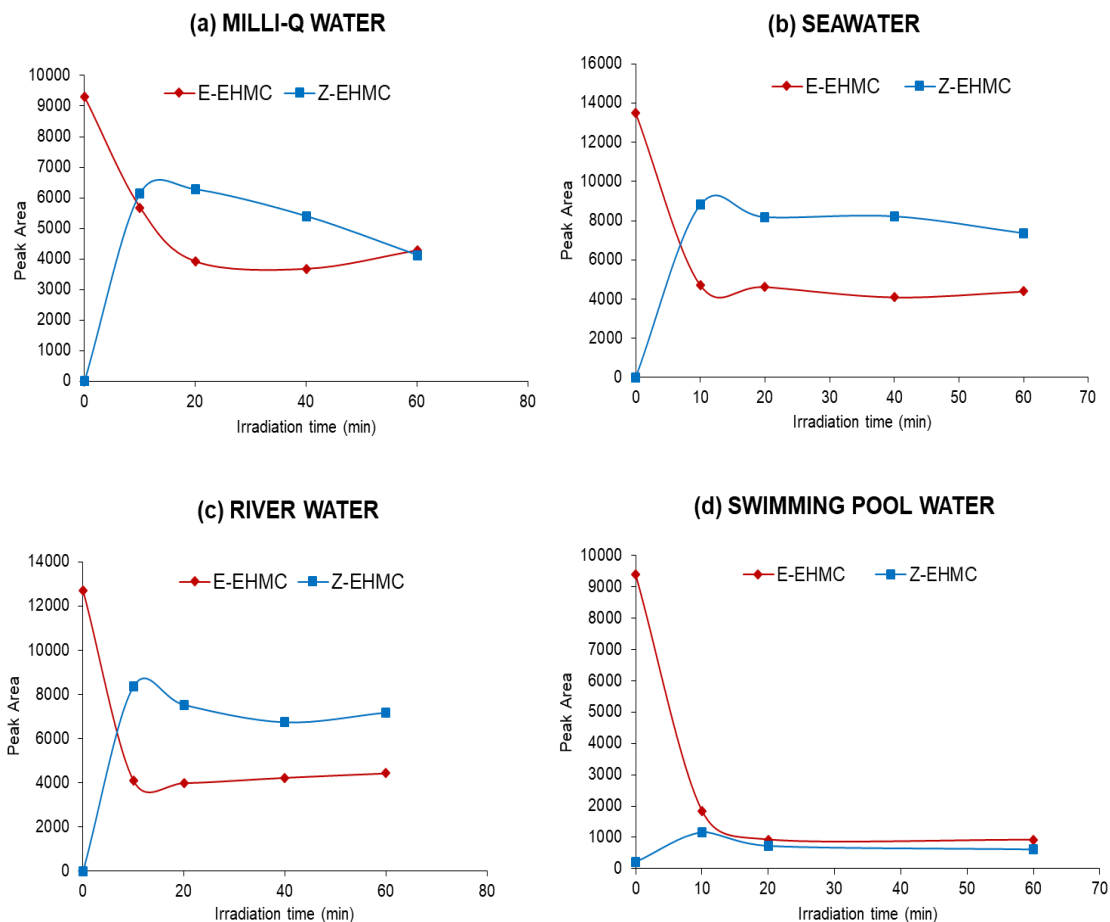


Figure 2. Graphics showing the peak area of undegraded E-EHMC and the peak area of generated Z-EHMC for the different water samples: (a) milli-Q water (b) seawater (c) river water (d) swimming pool water.

The results are quite different for the swimming pool water (see Figure 2). In this case, a significant reduction in signal was already observed after 10 minutes of UV exposure, along with the formation of the Z isomer, although both isomers exhibited very weak signals. In this matrix, the presence of chlorine radicals, hypochlorous acid, and other reactive oxygen species may promote additional chemical reactions with organic compounds. These findings were in consonance with the findings by Gackowska et al. (Gackowska et al., 2014; Gackowska et al., 2018), in which studied photodegradation of EHMC in the presence of reactive oxygen and chlorine species, using water medium. It reports that EHMC undergoes significant degradation when exposed to UV light in the

presence of hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl), leading to oxidation and chlorination products.

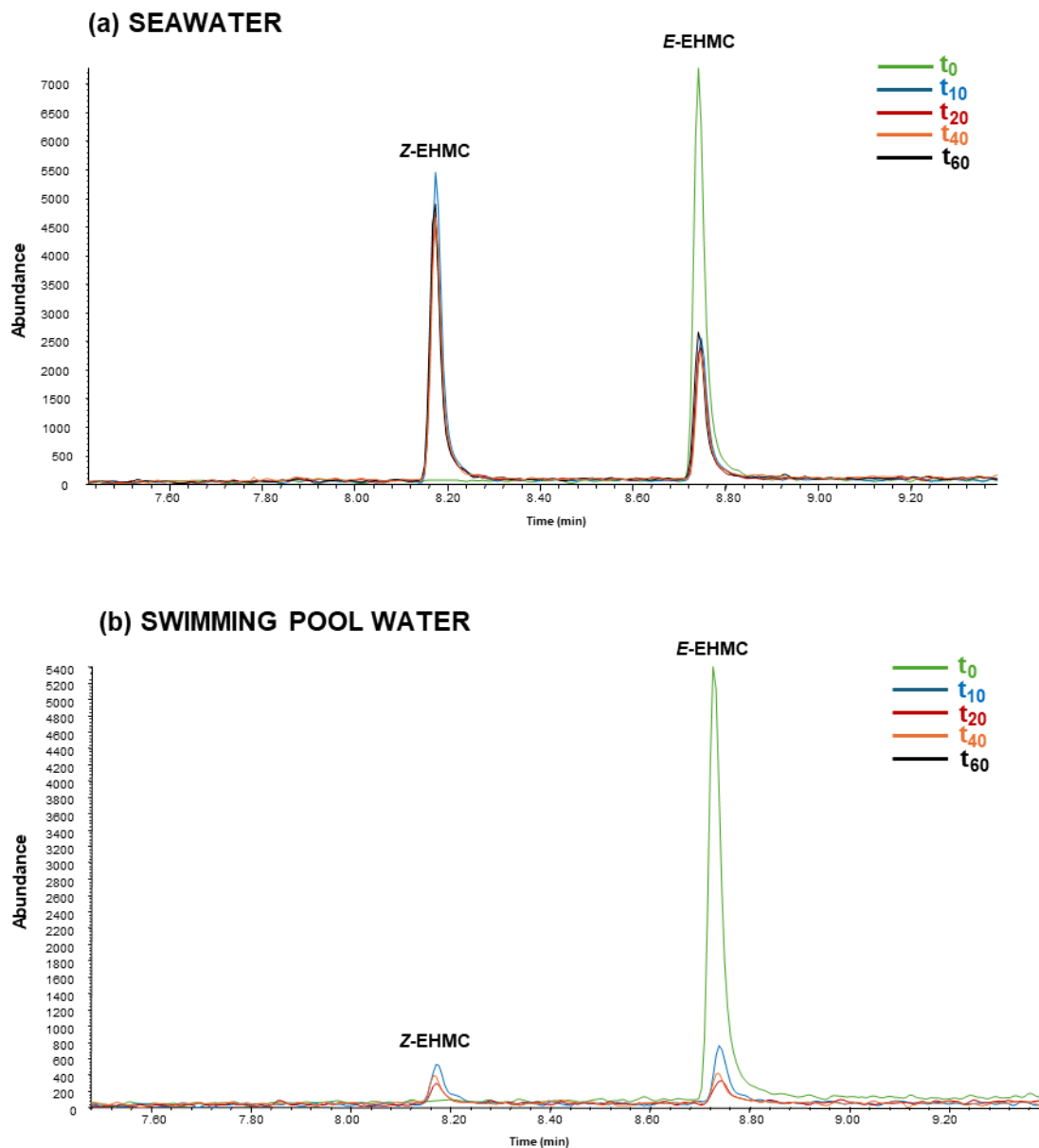


Figure 3. SPME-GC-MS ion chromatograms (ion m/z 178) of seawater and swimming pool water samples showing the formation of Z-EHMC and the decay of E-EHMC.

2.3.2. Photoisomerization of EHMC in diverse cosmetic products

To investigate the environmental origin of Z-EHMC isomer, several real (non-spiked) cosmetic samples containing EHMC were analyzed. The selected samples represent different formulations and applications, including hair oil, lip balm, and sunscreen cream. The hair oil, and lip balm had been previously used before analysis by the consumers, whereas the sunscreen cream was used exclusively for this study.

Quantitative analysis was performed to determine the concentration of EHMC in each sample. Given the typically high concentrations of EHMC in cosmetic formulations, a small amount of sample (5-8 mg) was weighed and homogenised in 4 mL of solvent using vortex mixing followed by ultrasonic agitation. An aliquot of the resulting solution was then diluted and analysed by GC-MS. As expected, in all cases, only the *E*-EHMC isomer was detected, with no observable presence of *Z*-EHMC, indicating that *Z*-EHMC is not inherently present in the original cosmetic formulations, even though two of the three samples had been previously used before this study and were acquired 1-2 years before. Regarding their concentrations, both the sunscreen cream and lip balm contained 57 mg g⁻¹ of EHMC, while the hair oil contained 8.6 mg g⁻¹. These values are below the maximum concentration established by the SCCS Opinion on Ethylhexyl Methoxycinnamate, which considers EHMC safe for use in cosmetic products at levels up to 10 % (100 mg g⁻¹) (Bernauer et al., 2025).

The three cosmetic products were applied to a glass slide (thin layer) to study the photodegradation of the cosmetic products itself and to an artificial skin model, in order to simulate real use conditions. The samples were then irradiated with UV light and natural sunlight. The detailed experimental procedure is described in Section 2.

2.3.3. Photoisomerization on glass slide

The cosmetic samples were irradiated for one hour under UV light in the photoreactor. To simulated even more environmental conditions direct sun irradiation was performed exposing the plates to natural sunlight for the same time (1 h). In parallel, a dark control test was performed to confirm that the possible formation of *Z*-EHMC was only due to UV and/or sun exposure; as expected *Z*-EHMC was not detected in the samples kept in the dark. The *cis* isomer was not found in the *t*₀ assays. In all the other cases the *cis* isomer was present in all samples after the exposure time for both UV lamp and sunlight at very high concentrations. The predominant isomer was not the same for the 3 samples. In the case of the sunscreen cream, the transformation reaction occurs in a lesser extent and the *trans* isomer is the predominant one representing about 70 % of the total. In the case of the lip balm also the *trans* is in general more abundant, but the differences between isomers are minor. On the other hand, the oil showed fastest transformation with similar signal for both isomers after sun exposure and higher *cis* response in other cases, those corresponding to UV exposure. For the three samples the sun exposure presented a

slightly lesser transformation but in general the results with both lights are quite parallel (Figure 4).

As commented, the samples were quite diverse in both appearance and purpose. Regarding EHMC content, the oil has a lower concentration (about 7 times less). Considering that the oil is a fluid and the concentration is minor could be logical to expect a faster EHMC transformation.

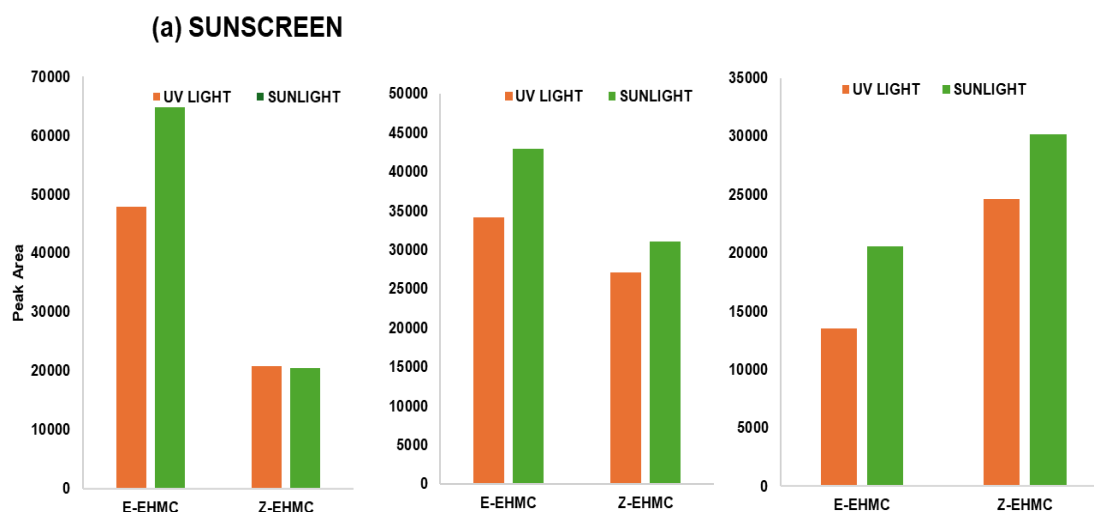


Figure 4: Comparison of results after UV light and real sunlight exposure (1 h) for the (a) Sunscreen cream (b) Lip balm, and (c) Hair oil.

2.3.4. Photoisomerization on artificial skin treated with cosmetic products

To simulate even more a real scenario, the three cosmetic products were applied on *in vitro* human skin. After skin conditioning, the procedure was very similar to the one followed previously (see also section 2). The skin was irradiated for 1 h and 2 h in the photoreactor. After skin irradiation, the *cis* isomer was again detected in all the samples. The results are shown in Figure 5. It can be seen that the results were quite parallel to those observed in plate. For the suncream the transformation occurs in a less extent with about 25 % formation of *cis* isomer. The reaction occurs more rapidly in the other matrices, especially in the hair oil where the concentrations for both isomers were similar. Regarding the exposure time, the results were also quite equivalent after 1 and 2 hour confirming the steady state reached in other samples and commented in literature. Figure 6 shows the chromatograms obtained for each one of the samples before and after irradiation (Irradiation time 1 h) were the chromatographic peaks for both isomers are clearly presented in the irradiated samples.

Table 1. Percentage of undegraded *E*-EHMC and generated *Z*-EHMC under controlled UV exposure on cosmetics *in vitro* human skin: (a) sunscreen, (b) lip balm, and (c) hair oil.

Irradiation time (min)	Sunscreen		Lip balm		Hair oil	
	<i>E</i> -	<i>Z</i> -	<i>E</i> -	<i>Z</i> -	<i>E</i> -	<i>Z</i> -
0	100	0	100	0	100	0
30	88	12	78	22	47	53
60	78	22	63	37	48	52
120	70	30	58	42	46	54

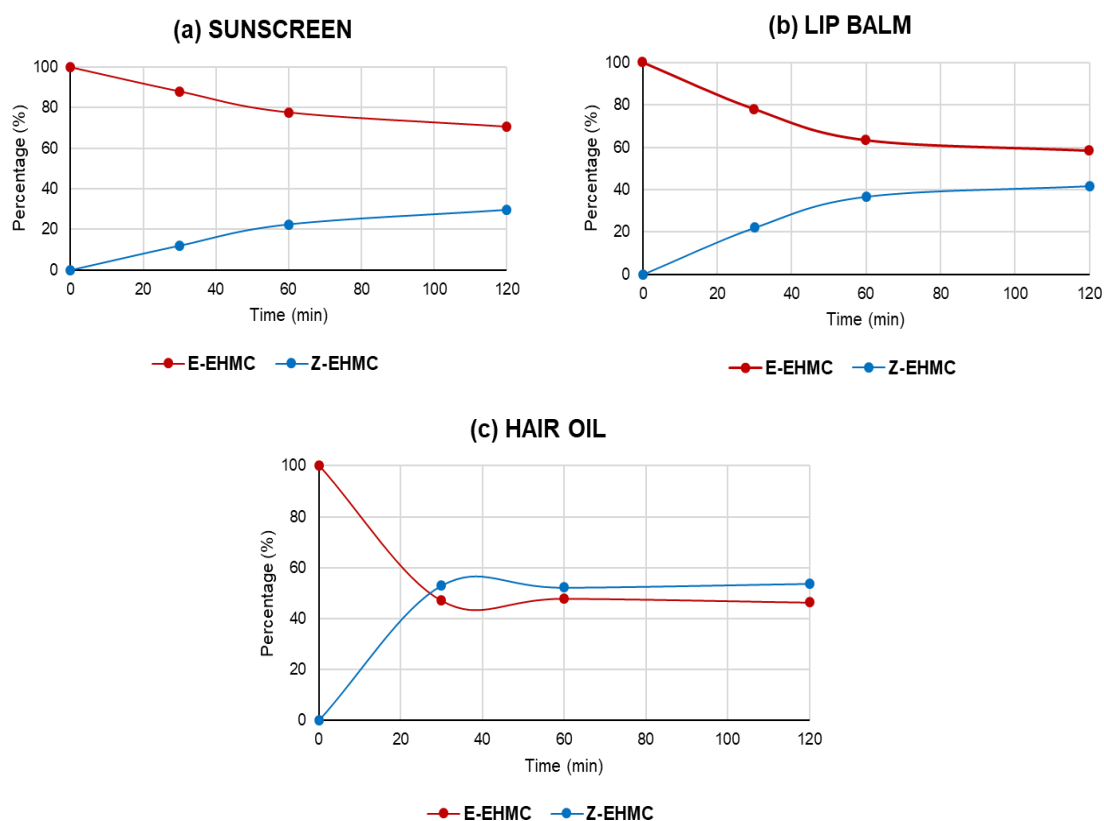


Figure 5: *E/Z* photoisomerization kinetics under controlled UV light exposure (a) sunscreen cream (c) lip balm (d) hair oil.

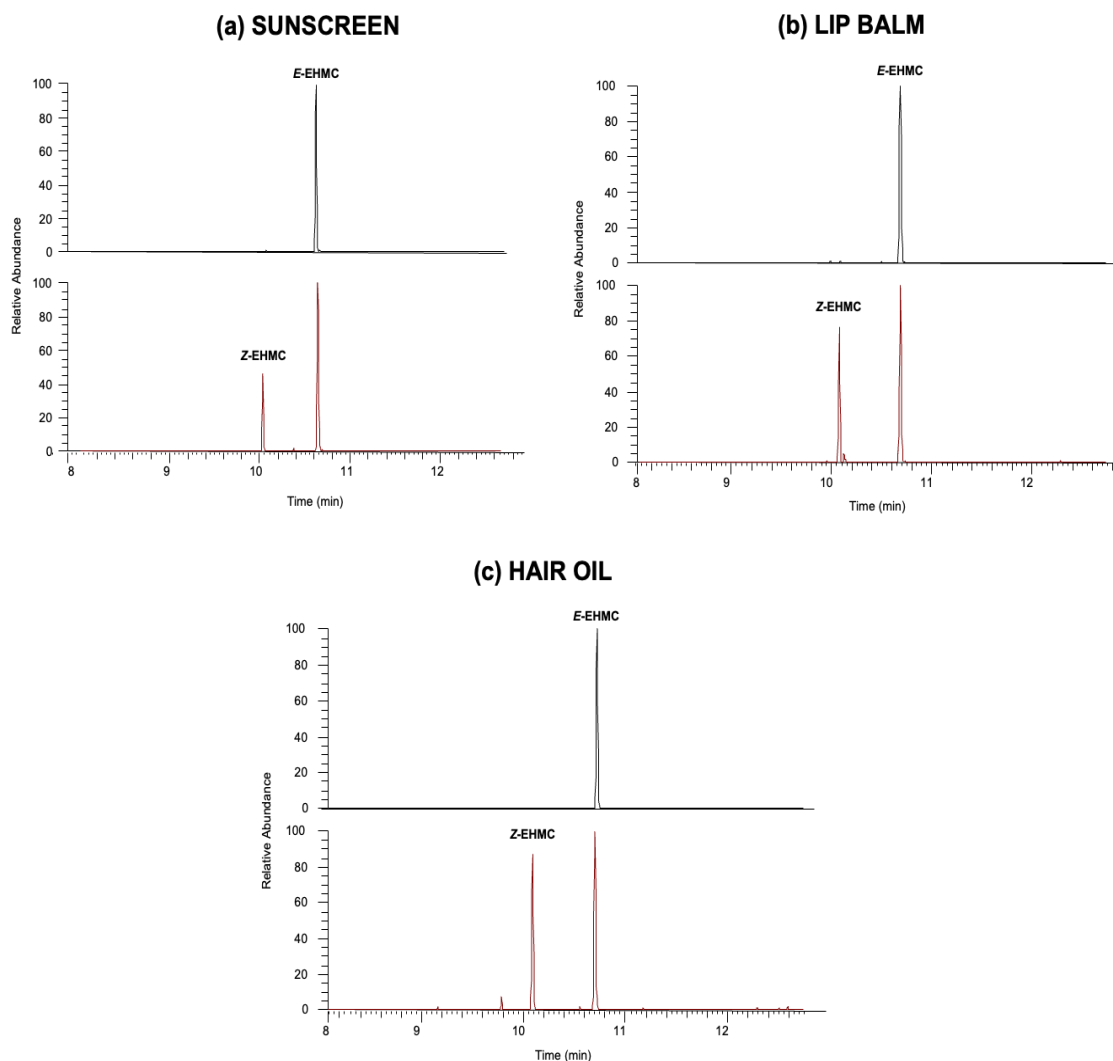


Figure 6: Chromatograms obtained for each one of the samples before and after irradiation (irradiation time 1 h).

Photodegradation was also study employing real sunlight. In this case, the exposure times were higher. The atmospheric conditions were cloudy day, high humidity and mean temperature 20 °C. The results shown in Figure 7 were again quite similar to the ones obtained by UV light exposure in the photoreactor and quite identical for both exposure times (30, 180, and 240 min).

Table 2. Percentage of undegraded *E*-EHMC and generated *Z*-EHMC under controlled UV exposure on cosmetics *in vitro* human skin: (a) sunscreen, (b) lip balm, and (c) hair oil.

Irradiation time (min)	Sun cream		Lip balm		Hair oil	
	<i>E</i> -	<i>Z</i> -	<i>E</i> -	<i>Z</i> -	<i>E</i> -	<i>Z</i> -
0	100	0	100	0	100	0
30	92	8	74	26	47	53
180	81	19	57	43	47	53
240	69	31	57	43	54	46

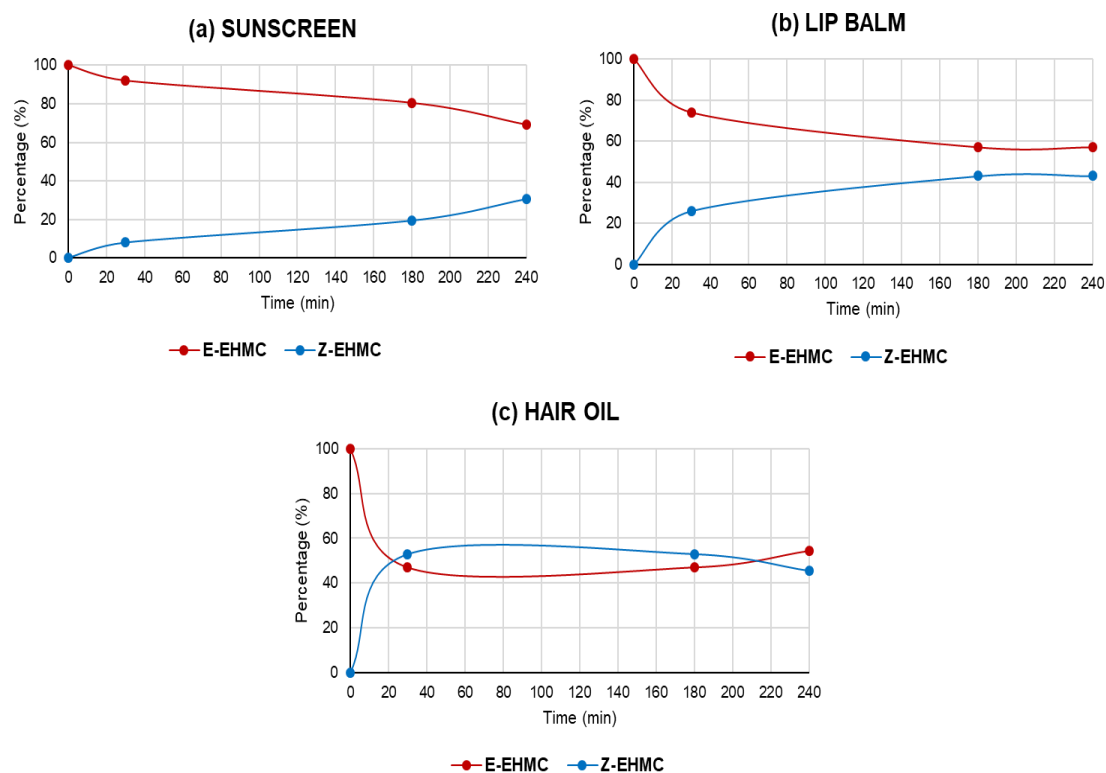


Figure 7: *E/Z* photoisomerization kinetics under controlled sunlight exposure (a) sunscreen cream (c) lip balm (d) hair oil.

2.4. Conclusion

This study demonstrates that EHMC undergoes rapid *E/Z* photoisomerization under realistic exposure conditions in both environmental and dermatological scenarios. GC-MS was employed to study the photoisomerization behavior in ultrapure, river, seawater, and swimming pool water. In aquatic environments, sample extraction behaviors were tracked at ng mL⁻¹ levels using rapid headspace SPME coupled to GC-MS. Photostationary state achieved within minutes and poor overall EHMC elimination observed in all waters, except swimming pool water demonstrated substantial degradation of both isomers, attributed to reactions with reactive chlorine species and oxidants present in chlorinated water. These findings indicate that EHMC persists in natural aquatic environments, while chlorinated recreational waters facilitate more degradation. In cosmetic products and to an *in vitro* human skin model only *E*-EHMC was initially detected in the commercial matrices (all within regulatory concentration limits 10% in the EU), and no *Z*-EHMC detected. After applying commercial formulations (sunscreen cream, lip balm, hair oil) to glass slide and to the skin model and irradiating them, GC-MS and GC-MS/MS confirmed rapid formation of *Z*-EHMC and evolution toward an *E/Z* photostationary state. In sunscreen cream, the *E*-isomer still represented ~70-80% of total EHMC, whereas in hair oil the *Z*-isomer became comparable to the *E*-isomer, showing more extensive transformation.

These results indicate that EHMC phototransformation is not an artefact laboratory irradiation but a systematic outcome of real-world exposure. This means consumer directly and quickly exposed to *Z*-EHMC, implying both reduced photoprotection and increased biological risk on skin. Prior regulatory evaluation of EHMC focus largely on the parent compound concentration limits in cosmetics (10% in EU) but generally do not account for on-skin photochemistry, environmental persistence, or isomer-specific toxicity. By showing that *Z*-EHMC forms rapidly, persists in a near photostationary state, and can enter aquatic systems at low (ppb) levels. Our results argue that of EHMC should no longer treat it as a single static molecule, but as a dynamic system of isomers and transformation products relevant to both environmental exposure and human health assessment

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3. Development of an Analytical Method Based on Derivatization Solid-Phase Microextraction Combined with Gas Chromatography-Mass Spectrometry for the Determination of Bisphenol Analogues in Environmental Waters

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3.1. Introduction

Bisphenols (BPs) are widely utilised as additives across numerous industries, such as plastic packaging, electronics, medical equipment and composites materials (Yang et al., 2025). Bisphenol A (BPA) and its analogues including BPAF, BPAP, BPB, BPF, BPS, and BPP, constitute a class of structurally related chemicals characterized by two phenolic rings connected by a bridging carbon atom or alternative linkages (Chen et al., 2016; Peng et al., 2020). BPA is the most extensively produced and used bisphenol analogue globally, with China being the leading contributor to the global market, accounting for approximately 3.3 million tonnes of BPA consumption in 2022 (Wang et al., 2024). BPA is recognised as an endocrine-disrupting chemical (EDC) and has been linked to numerous adverse health effects, including diabetes, cardiovascular disease, reproductive disorders, breast cancer and birth effects (Chen et al., 2025; Vom Saal et al., 2024). Growing concerns regarding widespread human exposure and its potential health impacts have prompted the implementation of regulations on BPA production and use. The U.S. Environmental Protection Agency (EPA) is continuing to evaluate BPA and its alternatives under the Toxic Substance Control Act (TSCA) to determine their safety profiles U.S. Environmental Protection Agency (European Chemical Agency, 2010). Regulatory agencies such as European Chemicals Agency (ECHA) have proposed restrictions under the REACH regulations on BPA and certain analogues, emphasising precautionary measures due to their endocrine disrupting potential (U.S. Environmental Protection Agency, 2022). In 2011, the European Union prohibited the use of BPA in infant feeding bottles (Fox et al., 2011). Likewise, in 2010, Canada banned the importation and sale of polycarbonate bottles containing BPA (Kiss, 2014). Regarding the regulations in water, the European Drinking Water Directive, from 12 January 2026, water suppliers will be required to meet a maximum BPA concentration of $2.5 \mu\text{g L}^{-1}$ (European Union, 2020).

Growing public concerns and regulatory restrictions on BPA have recently encouraged the production and use of BP analogues as substitutes for BPA in a range of applications (Česen et al., 2018; Zhang et al., 2019). Alternative bisphenols were initially considered less toxic than BPA. However, recent evidence suggests that BPAF, BPF, BPB, BPS, and tetramethyl bisphenol F (TMBPF) may exerts adverse effects that are comparable to or even greater than those of BPA (Hyun et al., 2021; Qiu et al., 2021). Alternative bisphenols were initially considered less toxic than BPA. However, recent evidence suggests that BPAF, BPF, BPB, BPS, and tetramethyl bisphenol F (TMBPF) may exerts adverse effects that are comparable to or even greater than those of BPA (Hyun et al., 2021; Qiu et al., 2021; Reininger & Oehlmann, 2024; Rifa et al., 2025). Given their harmful effects on humans and wildlife including genotoxicity, estrogenicity, cytotoxicity, and carcinogenicity, these compounds have been classified as contaminants of emerging concern (Wang et al., 2021).

Due to their extensive use in essential applications ranging from electronics to food packaging, substantial quantities of these chemicals have been released into the aquatic environment (Erjavec et al., 2016). BPA can migrate from resins or polymeric materials into water or food when exposed to elevated temperatures. Consequently, the likelihood of BPA contamination in these matrices is considerable, given the widespread consumption of drinking water and canned foods globally (Kamalabadi et al., 2016). Because BPs are typically present at very low levels in water and food samples, directly detecting them is difficult, and therefore sample preparation methods with high sensitivity and selectivity are essential to effectively extract and concentrate these compounds while simultaneously eliminating major interferences before the instrumental analysis (Rozaini et al., 2017).

Traditional methods tend to be labour-intensive and time taking, consuming large amounts of both samples and organic solvents, as multiple extraction steps are usually required to achieve complete isolation of various BPs. The extensive use of organic solvents contribute to environmental pollution, poses health risk, and incurs additional costs for waste treatment (Viñas et al., 2010). Consequently, in recent years, modern and miniaturised extraction techniques have been adopted, enabling reductions in sample and solvent volumes, minimising waste generation, and aligning with the principles of ‘Green Chemistry’. Different sample preparation techniques both conventional and modern such as solid-phase extraction (SPE) (Česen et al., 2018; Gao et al., 2023; Zainuddin et al.,

2023; Zhang et al., 2019), ultrasound-assisted extraction (UAE) (Kiejza et al., 2022), dispersive liquid-liquid microextraction (DLLME) (Rocha et al., 2016), and liquid-liquid extraction (LLE) (Wang et al., 2018), have been reported in the literature for extracting BPs from various types of water matrices of environmental concerns. Solid-phase microextraction (SPME) represents an effective alternative to conventional methods, as it provides a solvent-free and more environmentally sustainable alternative to previously described procedures. Moreover, SPME generally yields a high enrichment factor, shortens extraction time, and can be easily automated, facilitating high sample throughput (Vila et al., 2018).

Advances in instrumental technologies, particularly chromatographic techniques like liquid chromatography (LC) and gas chromatography (GC) chosen according to the chemical properties of the target compounds and their coupling with mass spectrometry (MS) or tandem mass spectrometry (MS/MS), have been extensively applied in the analysis of BPs. Nevertheless, effective sample preparation remains essential, as it enables the extraction, pre-concentration, and clean-up of analytes from complex matrices, thereby improving method sensitivity and prolonging instrument lifespan. Since BP analogue generally exhibit low volatility, derivatisation is required to enhance their volatility prior to GC-MS analysis (Maciel et al., 2019; Owczarek et al., 2022; Wang et al., 2017). The integration of SPME with GC-MS offers a powerful analytical approach, and SPME is therefore considered a promising technique for extracting BPs from environmental water samples.

This study seeks to establish a rapid, sensitive, and selective approach for analysing BP compounds in drinking waters, environmental waters, and wastewater samples using *in-vial* derivatisation SPME, without relying on organic solvents, and combined with GC-MS detection in selected ion monitoring mode (GC-MS-SIM). Linearity, precision, and detection limits were assessed to evaluate the optimised method, intended for the analysis of these compounds in water samples collected from various locations in Spain and Portugal. To the best of our knowledge, this is the first reported SPME-GC-MS method employing simultaneous *in-vial* acetylation for the analysis of these compounds in environmental waters.

3.1. Experimental

3.1.1. Chemicals and Materials

A standard mixture containing BPs (BPAF, BPF, BPE, BPA, BPG, BPC2, BPZ, and BPM), each at a concentration of 2000 $\mu\text{g mL}^{-1}$ in acetone, was sourced from LGC Standards Ltd. (Barcelona, Spain) with a minimum purity of 98%. BPB (97.5 %) was purchased from TargetMol (Boston, USA). Acetic anhydride, used as the derivatising agent, was obtained from Merck (Darmstadt, Germany), whereas N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, 99%) containing 1% trimethylchlorosilane (TMCS) was procured from Supelco (Bellefonte, PA, USA). Pyridine (99.8%) was provided by Sigma-Aldrich. Methanol and ethyl acetate were purchased from Fluka Analytical (Steinheim, Germany), while ultrapure water (MS-grade water) was acquired from Scharlab (Barcelona, Spain). Sodium chloride (NaCl) was obtained from Panreac (Barcelona, Spain), and sodium bicarbonate (NaHCO_3 ; 99.0-100.5%) from Glentham Life Sciences Ltd. (SN13 9SW, United Kingdom). All chemicals and solvents used were of analytical grade.

Commercially available SPME fibres, 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS), 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS/DVB), and 85 μm polyacrylate (PA), mounted in manual holders, were obtained from Supelco (Bellefonte, PA, USA). Prior to use, the fibres were prepared according to the provided guidelines by placing them into the GC-MS injection port under a continuous carrier gas flow, with DVB/CAR/PDMS at 270 $^{\circ}\text{C}$ for 1 hour, PDMS/DVB maintained at 250 $^{\circ}\text{C}$ for 30 minutes, PA at 280 $^{\circ}\text{C}$ for 1 hour, and PDMS at 250 $^{\circ}\text{C}$ for 1 hour. A stock solution containing all the BP standards was prepared in methanol. Working solutions were subsequently prepared and stored at -20 $^{\circ}\text{C}$.

3.2.2. Sampling sites and sample collection

Various real water samples were obtained from locations in Galicia (NW Spain) and Porto (Portugal): river water, seawater, wastewater, port water, artificial turf crumb rubber football field irrigation water, tap water, spring water and swimming pool water. All these samples were collected (30 mL - 1 L) in glass vials or bottles, transported and stored at -20 $^{\circ}\text{C}$ in the dark. Further details of the real samples are provided in Table 1.

Table 1. Detailed description of the 19 analyzed samples			
Code	Description and Location	Sampling date (DD/MM/YY YY)	Type of sample
MQ	MiliQ water from laboratory system, USC*, Spain	24/04/2025	Mili Q water
SPW1	Drinking water (Lab), USC, Spain	15/04/2025	Spring water
SPW2	CABREIROA, Aqua mineral natural de Galicia, Spain	24/04/2025	Spring water (bottled)
SPW3	Drinking water (Lab), USC, Spain	28/05/2025	Spring water (bottom of water can 50 L)
WW1	Wastewater sampled after sludge centrifugation, Porto, Portugal	04/07/2024	Wastewater
RW	Sarela River, Santiago, Spain	21/04/2025	River Water (RW)
TW1	Tap water from lab, Santiago, Spain	25/04/2025	Tap water
TW2	Tap water from house, Santiago, Spain	26/05/2025	Tap water
ICW	Tap water from house, Santiago, Spain	26/05/2025	Ice cube water from ice tray
SP1	Santiago, Spain		Swimming pool water
SP2	Vilanova da Cerveira, Portugal	08/11/2024	Swimming pool water
SW1	Sea water taken several km far from Galicia coast, Spain	08/04/2025	Seawater
SW2	Sea water from beach, Vilagarcía, Spain	11/04/2025	Seawater
SW3	Sea water from beach, Boiro, Spain	19/01/2025	Seawater
FW1	Campus USC, Santiago, Spain	27/06/2024	Artificial turf crumb rubber football field irrigation water
FW2	Campus USC, Santiago, Spain	27/06/2024	Artificial turf crumb rubber football field irrigation water
PW1	Port water, Vigo Spain	27/04/2025	Port water
PW2	Port water, Ferrol Spain	26/04/2025	Port water
PW3	Galician Estuary, Ferrol, Spain	26/04/2025	Port water

3.2.3. Solid-phase microextraction procedure

Following the optimised experimental conditions (see Results and Discussion), 15 mL of water samples were placed in 20 mL vials. To perform *in-vial* derivatization, 100 mg of NaHCO₃ and 50 µL of acetic anhydride were introduced into the mixture. The vials were sealed with aluminium caps fitted with PTFE-faced septa and placed in a water bath maintained at 100 °C. After 5 minutes to allow the sample to reach a steady state, the SPME fibre (DVB/CAR/PDMS) fibre was inserted directly into the sample (DI-SPME) and exposed for 30 minutes. The samples were continuously stirred throughout the extraction employing a magnetic stirrer plate (Agimatic-N with heating, J.P. SELECTA®), and a magnetic bar. After extraction the fibre was retracted into the SPME holder needle transferred to the GC injection port, and thermally desorbed for 5 minutes at 270 °C.

Ultrapure water standards were prepared by spiking with the target compounds to obtain various concentrations and analysed using the aforementioned SPME procedure. To avoid false-positive results, fibre blanks and procedural blanks using 15 mL of ultrapure water were carried out each day.

3.2.4. GC-MS analysis

Analysis were conducted on an Agilent 7890A gas chromatography coupled to an Agilent 5975C inert mass selective detector featuring a triple-axis detector, alongside an Agilent 7693 autosampler (Agilent Technologies, Palo Alto; CA, USA). A ZB-Semivolatiles column (30 m × 0.25 mm × 0.25 µm) from Phenomenex (Torrance, CA, USA) was employed for chromatographic separation. High purity helium (99.999%) served as the carrier gas at a flow rate of 1.0 mL min⁻¹. The oven temperature programme began at 80 °C (held for 1 minute) to 210 °C (held for 2 minutes) at 15°C min⁻¹, and then to 280 °C (held for 1 minute) at 3 °C min⁻¹, with a total run time of 34 minutes. The injection volume was 1 µL for the organic solvent standards, and was performed in pulsed splitless mode (1 min at 75 mL min⁻¹) at temperature of 260 °C. The transfer line, ion source and quadrupole were maintained at temperatures of 290, 150, and 300°C, respectively. Compounds were examined in selected ion monitoring (SIM) mode, as summarised in Table 2.

Table 2. Experimental GC–MS parameters for the analysis of the target BP compounds.

Compounds	Abbreviation	CAS number	MW (g mol ⁻¹)	Retention Time	Ions*
4,4'-(Hexafluoroisopropylidene)diphenol	BPAF	1478-61-1	336.23	13.86	<u>267</u> , 336, 378
4,4'-Methylenediphenol	BPF	620-92-8	200.23	16.80	107, <u>200</u> , 242
4,4'-Ethylidenebisphenol	BPE	2081-08-05	214.26	17.64	<u>199</u> , 214, 256
4,4'-Isopropylidenediphenol	BPA	80-05-7	228.29	18.53	<u>213</u> , 228, 270
4,4'-(1-Methylpropylidene)bisphenol	BPB	77-40-7	242.31	20.28	<u>213</u> , 119, 214
4,4'-(Propane-2,2-diyl)bis(2-isopropylphenol)	BPG	127-54-8	312.4	20.53	<u>297</u> , 312, 339, 354
1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethylene	BPC2	14868-03-2	281.1	23.00	210, <u>280</u> , 322
4,4'-(cyclohexane-1,1-diyl)diphenol	BPZ	843-55-0	268.3	27.34	<u>225</u> , 268, 310
4,4'-(1,3-phenylenediisopropylidene)bisphenol	BPM	13595-25-0	346.5	32.47	<u>331</u> , 373, 388

*Quantification ions are underlined

**m/z corresponding to the acetylated BP derivatives are denoted in italics

3.2.5. Statistical analysis

Descriptive and basic statistical analyses, as well as evaluation of the experimental design, were carried out using the Statgraphics Centurion XIX software package (Manugistics, Rockville, MD, USA). A factorial design was selected to optimise the extraction step by assessing the experimental parameters, including the interaction effects among factors. The greenness and practicability of the proposed method were evaluated with the AGREEPrep and the BAGI (Blue Applicability Grade Index) softwares. All calculations for method validation were performed using Microsoft Excel.

3.3. Results and discussion

3.3.1. Method optimization

The GC method was optimised by systematically assessing various instrumental parameters, including the oven temperatures gradient, injection mode, injection volume, and carrier gas flow rate. The optimised conditions detailed in Section 2.4 (GC-MS analysis) yielded better sensitivity and selectivity while reducing the total analysis time.

3.3.2. Selection of derivatizing agent

In the preliminary experiments, both silylation and acetylation of the target analytes were evaluated. Given the presence of an active hydrogen atom in the -OH groups of these molecules, such derivatization approaches are considered appropriate. As bisphenol analogues typically exhibit low volatility, derivatization is required to enhance their volatility prior to GC-MS analysis (Hernando et al., 2004).

For acetylation, BP standard solution in ethyl acetate were treated with 10 μ L of pyridine and 60 μ L of acetic anhydride, and incubated at 60 °C for 60 minutes. In the silylation approach, the same volume (60 μ L) of the silylating reagent BSTFA was used under identical time and temperature conditions. Both derivatization approaches yielded comparable chromatographic quality with higher sensitivity and better chromatographic peak shapes for all the compounds, in comparison to the non-derivatized BPs, some of them not even detected without derivatization. Silylation produced slightly higher responses. However, this procedure cannot be carried out in water (Carro et al., 2024).

Generally, silylation needs temperature control, a time to complete the process, and an anhydrous medium while acetylation can occur in water, which valued a green solvent (Wang et al., 2018). Besides, the derivatizing reagent for silylation is more toxic and expensive than acetic anhydride. In addition, some by-products may be produced under silylation due to the elevated reaction temperature (Li et al., 2001). Given these conditions and the goal of studying bisphenol compounds in various water samples acetylation was selected as the preferred derivatization method.

3.3.3. Optimization of the SPME extraction process

The sensitivity of the SPME extraction technique is predominantly influenced by the distribution constant of the analytes between the sample matrix and the fibre material. It is crucial to consider not only the individual effects of the factors that influence the extraction, but also the interaction effects between them. Accordingly, optimization was

conducted to determine both the relevant factors and their optimal levels, considering a multivariate experimental design. The fibre coating constitutes one of the most critical parameters, as the extraction efficiency of the target compounds depends on their affinity for the fibre material. To identify the most suitable fibres to include in the experimental design, preliminary tests were carried out using DVB, PA, CAR/PDMS/DVB, and PDMS/DVB fibres. These experiments were performed with 15 mL of ultrapure water spiked to $0.1 \mu\text{g L}^{-1}$ of the compounds, with the addition of 100 mg NaHCO_3 and 200 μL acetic anhydride, under immersion mode at 100°C for 30 minutes. The results are presented in Figure 1a. Among these, the DVB/CAR/PDMS fibre exhibited the highest extraction efficiency for the majority of the target BP compounds, followed by PDMS. Both were selected for further optimization.

The extraction mode is another key parameter in SPME. Both headspace (HS) and direct immersion (DI) modes were evaluated using the most appropriate fibres, DVB/CAR/PDMS and PDMS. Experiments were carried out under the same conditions as previously described. The results for the DVB/CAR/PDMS fibres are shown in Figure 1b. As illustrated, higher responses were observed using the DI mode for all compounds, which can be attributed to the direct contact of the fibre with the sample, enhancing the transfer of analytes. Conversely, several compounds were not detected when HS mode was applied.

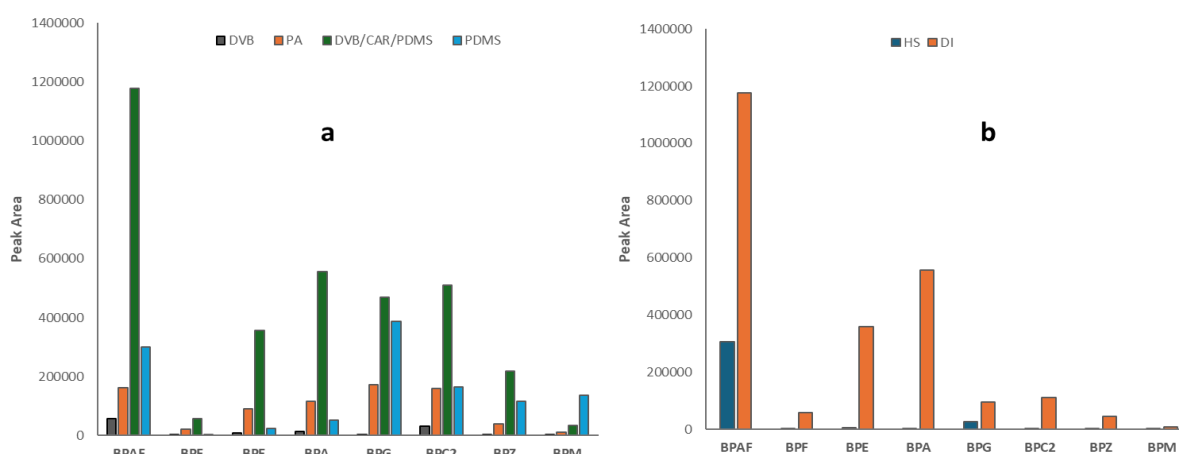


Figure 1. (a) Influence of fibre coatings in the extraction efficiency (responses for the last 4 compounds have been multiplied by 5 to facilitate visualization) and (b) extraction mode HS vs DI.

The efficiency of SPME depends on several factors, and when the extraction is combined with *in situ* derivatization, the yield of derivatization also affects the overall process. In addition to the fibre coating, the extraction mode, the volume of the derivatizing agent acetic anhydride and amount of NaHCO₃, and the extraction temperature should be studied. The addition of salt (NaCl), might improve extraction by raising the ionic strength of the solution, thereby reducing the solubility of organic compounds in water and facilitating their transfer to the fibre. However, this effect can vary depending on the analytes' polarity and molecular weight. Both presence and absence of NaCl were tested to determine its role in enhancing or inhibiting extraction efficiency. Another important factor was the quantity of NaHCO₃, which was added to buffer the pH and assist in the *in-vial* derivatization process. Alongside this, the volume of acetic anhydride, the chosen derivatizing agent, was varied to identify the amount that ensured extensive derivatization. All experiments were performed at elevated temperatures (up to 100 °C) to facilitate both derivatization and analyte transfer into the fibre and stirring was applied to reduce equilibrium time and enhance analyte fibre contact (Vila et al., 2016; Vila et al., 2017).

A screening factorial design was employed to simultaneously evaluate the effects of the five aforementioned factors. The addition of salt (Factor A) was tested at two levels (0 and 30 % w/v), the volume of the acetic anhydride (Factor B) at 50 and 200 µL, the amount of NaHCO₃ (Factor C) at 50 and 100 mg, the temperature (Factor D) at 60 and 100 °C, and the type of fibre (Factor E), using DVB/CAR/PDMS and PDMS. These factors, summarized in Table 3, were investigated using a 2⁵⁻¹ factorial design, resulting in 18 experiments, with one additional included for a total of 19 experiments. All experiments (n=2) were performed using 15 mL of ultrapure water spiked at 5 µg L⁻¹ with the target compounds, at 100 °C for 30 min.

Table 3. Experimental factors and levels in the experimental design.					
Factors	Code	Low (-)	High (+)	Units	Continuous
NaCl	A	0	30	%	Yes
AA*	B	50	200	μL	Yes
NaHCO ₃	C	50	100	mg	Yes
Temperature	D	60	100	°C	Yes
Fibre	E	DVB/CAR/PDMS	PDMS		No
* Acetic anhydride					

The analysis of variance (ANOVA), summarised in Table 4, illustrates the effects of the examined factors on the responses. The F-ratio reflects to which each factor and their interactions contribute to the variance of the response, while the p-values indicate the statistical significance of these effects. Factors or interaction with p-values < 0.05 are considered statistically significant at the 95% confidence level. As shown, the salting-out effect adding NaCl (%) and the fibre type were significant for most of the compounds, as was the interaction between these two factors (AE). Temperature was significant for certain compounds, whereas the remaining factors generally did not show significant effects.

Table 4. ANOVA table for the main factors and two-factors interactions obtained for each BP analogous. Values in bold and italic denote statistical significance (p-value < 0.05)

	A		B		C		D		E		AB		AC	
	Salting-out effect adding NaCl (%)		Acetic Anhydride (μ L)		NaHCO ₃ (mg)		Temperature (°C)		Fibre Type					
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
BPAF	2.34	0.1698	0.34	0.5805	0.22	0.6522	0.55	0.4820	4.35	0.0754	0.08	0.8034	1.02	0.3469
BPF	12.80	0.0090	0.01	0.9357	0.21	0.6596	0.12	0.7429	8.68	0.0215	0.01	0.9193	0.10	0.7608
BPE	14.94	0.0062	0.00	0.9897	0.59	0.4682	0.00	0.9844	8.25	0.0239	0.01	0.9220	0.05	0.8366
BPA	17.03	0.0044	0.00	0.9852	1.30	0.2924	0.48	0.5105	8.14	0.0246	0.07	0.8214	0.15	0.7367
BPG	459.69	0.0022	71.44	0.0137	232.82	0.0043	160.46	0.0062	229.32	0.0043	52.36	0.0186	192.33	0.0052
BPC2	5.21	0.0625	5.69	0.0544	0.01	0.9099	1.97	0.2098	2.94	0.1372	0.19	0.7023	0.13	0.7516
BPZ	1.95	0.2215	0.81	0.4103	2.94	0.1470	5.39	0.0679	5.18	0.0718	1.04	0.3552	1.56	0.2670
BPM	106.95	0.0092	17.48	0.0527	17.93	0.0515	29.62	0.0321	46.74	0.0207	0.45	0.5723	34.83	0.0275

Table 4. ANOVA table for the main factors and two-factors interactions obtained for each BP analogous. Values in bold and italic denote statistical significance (p-value < 0.05)

	AD		AE		BC		BD		BE		CD		CE		DE	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
BPAF	2.97	0.1285	11.21	0.0123	0.57	0.4741	0.02	0.9032	0.00	0.9784	0.94	0.3639	0.00	0.9552	0.04	0.8681
BPF	0.62	0.4561	0.02	0.8942	6.80	0.0350	0.03	0.8797	0.00	0.9533	0.02	0.9017	0.13	0.7255	2.16	0.1850
BPE	0.95	0.3627	3.07	0.1231	7.18	0.0316	0.06	0.8245	0.01	0.9494	0.03	0.8760	0.29	0.6063	2.82	0.1370
BPA	2.16	0.1854	10.09	0.0156	6.30	0.0404	0.13	0.7550	0.00	0.9538	0.03	0.8776	0.56	0.4789	3.04	0.1249
BPG	158.62	0.0062	224.49	0.0044	43.02	0.0225	64.59	0.0151	158.32	0.0063	141.25	0.0070	50.58	0.0192	52.97	0.0184
BPC2	2.53	0.1631	31.69	0.0013	2.51	0.1644	0.56	0.4839	0.02	0.9012	4.16	0.0875	0.13	0.7538	4.22	0.0858
BPZ	2.37	0.1842	26.11	0.0037	1.23	0.3183	0.08	0.8080	0.85	0.3983	0.15	0.7368	0.12	0.7613	2.47	0.1766
BPM	51.62	0.0188	12.19	0.0731	0.17	0.7224	0.12	0.7641	4.15	0.1785	17.63	0.0523	9.22	0.0935	1.01	0.4198

Figure 1 graphically shows the influence of the factors through the pareto charts and Figure 2b including the main effects plots for some selected compounds. As commented, the fibre coating (D) resulted statistically significant for most of the compounds (BPF, BPE, BPA, BPG and BPM), being more favourable the use of the DVB/CAR/PDMS for all analytes. The salting-out effect (A) was significant showing different behaviour regarding the compounds.

Analysing only the main factors (see Figure 2b) for BPF, BPE, and BPA, the salt addition improved extraction whereas for BPG and BPM the behaviour was just the opposite. Nevertheless, the interaction between those factors is significant in most cases. AE interaction shows as in general the addition of salt benefits PDMS extraction but disfavours the extraction by the DVB/CAR/PDMS fibre, as can be seen in the interaction plots in Figure 2c. Therefore, considering the more suitable fibre the addition of NaCl is not recommended. The temperature was also significant for 2 compounds but for all the targets higher temperature (100 °C) achieved higher response. Regarding acetic anhydride and NaHCO₃, in general were not significant for any compound except for BPG for which 50 µL and 100 mg, respectively, were more suitable. To conclude, the selected SPME conditions extracted from this experimental design were: 0% NaCl, 50 µL of acetic anhydride, 100 mg NaHCO₃, 100 °C, and DVB/CAR/PDMS fibre.

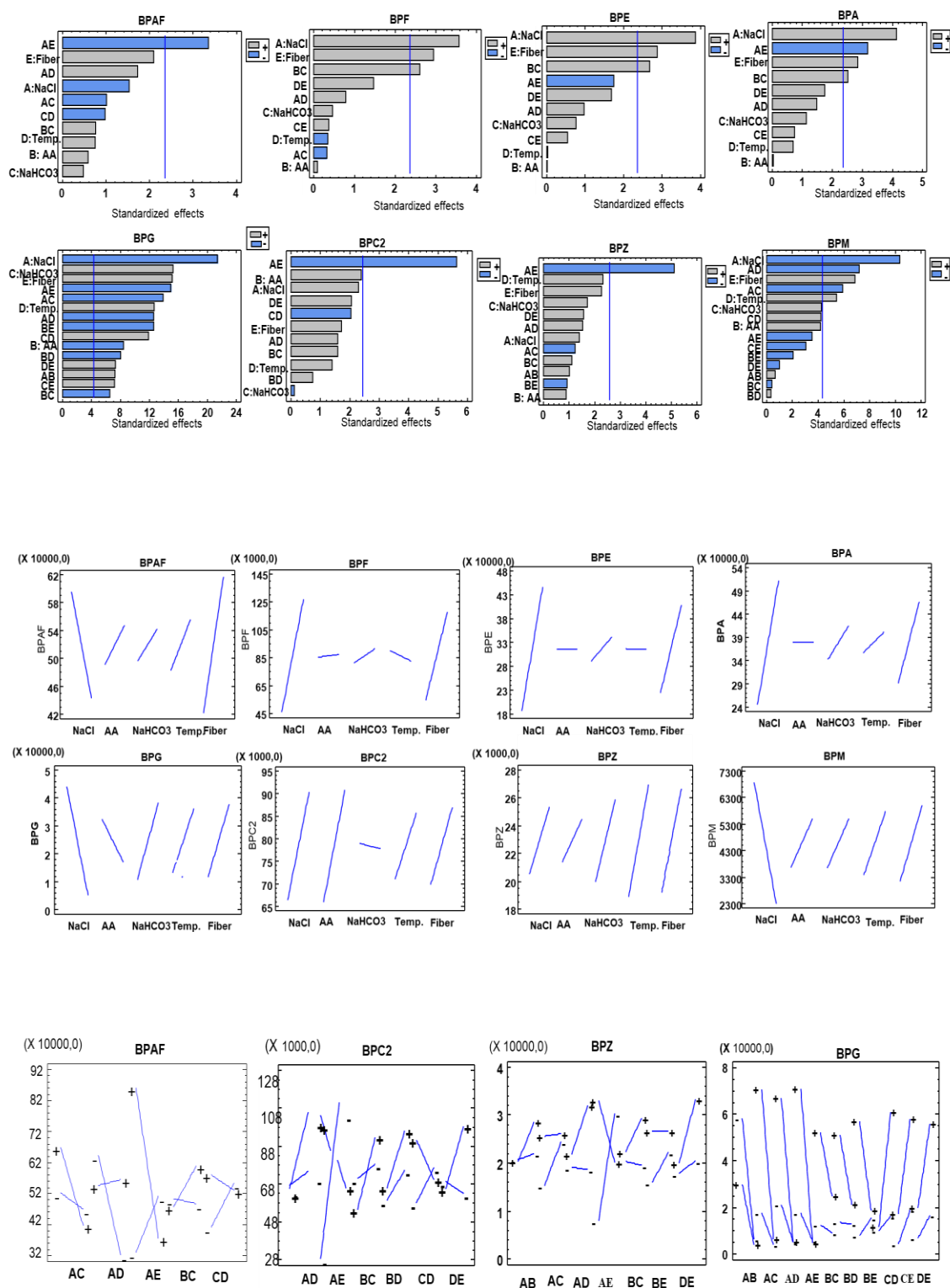


Fig. 2. Experimental design optimization: a) pareto charts b), main effects plots, c) interaction plots for the target compounds.

3.3.4. Method performance evaluation

The method performance was evaluated in terms of linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and accuracy. The results are presented in **Table 5**. Calibration was conducted using SPME under optimised conditions across a concentration range of 0.005 to 5 $\mu\text{g L}^{-1}$ for each analyte in ultrapure water, at seven levels with three replicates per level ($n=3$). The method demonstrated a directly proportional relationship between the amount of compound and its chromatographic response, with determination coefficients (R^2) equal to or greater than 0.999. **Figure 3** shows a SPME-GC-MS chromatogram of a sample (ultrapure water spiked at 1 $\mu\text{g L}^{-1}$).

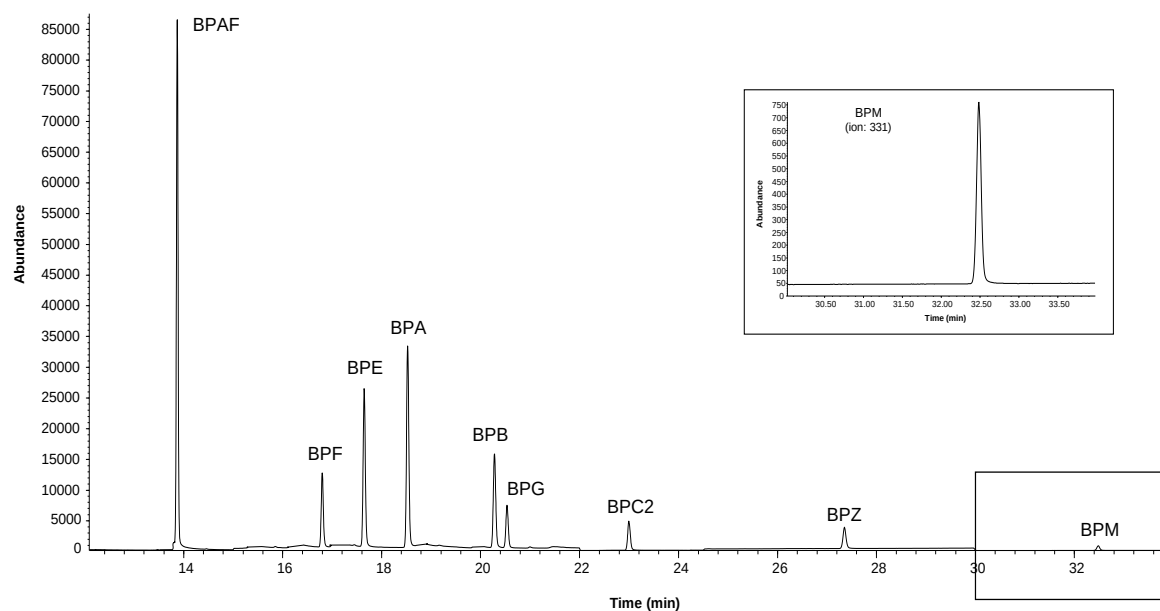


Figure 3. SPME-GC-MS chromatogram of a sample (ultrapure water spiked at 1 $\mu\text{g L}^{-1}$). For better visualization, the ion chromatogram for BPM is included.

The LODs and LOQs were determined as the analyte concentration giving signal-to-noise (S/N) ratio of 3 and 10, respectively, using spiked samples at low concentrations level. The limits were below 0.005 $\mu\text{g L}^{-1}$ for most analytes. The instrumental precision was assessed within a day ($n=3$) and across several days ($n=6$) (Table 5).

Table 5. Linearity, limits of detection and quantification, precision, recoveries and mean precision in real samples.

Compounds	Linearity		LOD	LOQ	Precision (RSD%)		Recoveries and mean precision (RSD) in real samples
	Range ($\mu\text{g L}^{-1}$)	R^2	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)	Intra-day n=3	Inter-day n=6	%
BPAF	0.005-5	0.9992	0.00013	0.00044	16	13	107 (11)
BPF	0.005-5	0.9995	0.00088	0.00292	14	10	99.7 (9.8)
BPE	0.005-5	0.9993	0.00134	0.00446	6.5	20	93.6 (8.9)
BPA	0.005-5	0.9994	0.00208	0.00694	7.9	12	98.6 (9.0)
BPB	0.005-5	0.999	0.00038	0.00126	7.8	12	90.5 (12)
BPG	0.005-5	0.9992	0.00042	0.00142	8.2	13	86.6 (20)
BPC2	0.005-5	0.9996	0.00040	0.00133	8.6	12	110 (6.7)
BPZ	0.005-5	0.9998	0.00130	0.00435	7.2	6.6	101 (6.4)
BPM	0.005-5	0.9994	0.00227	0.00500	9.8	13	97 (7.4)

Recovery studies were performed using four different real water matrices (spring, river, waste and seawater) spiked at $0.1 \mu\text{g L}^{-1}$ with the nine BPs. In those cases where the compounds were initially detected in the samples, their responses were subtracted. Figure 4 presents the results obtained for each of the water matrices. For the four real samples the recoveries for all compounds were satisfactory, ranging from 73% to 120%, as shown in Table 6.

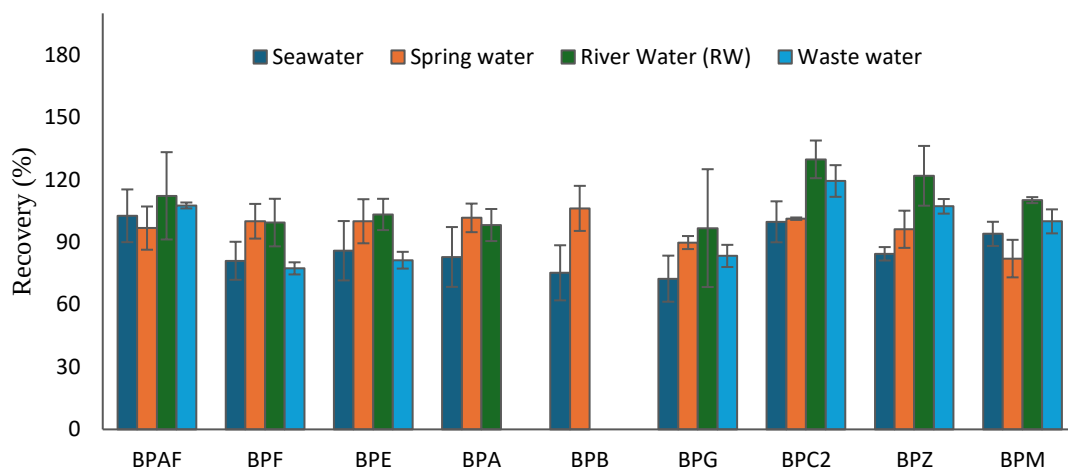


Figure. 4. Recoveries obtained for different water samples: seawater, spring water, river water and wastewater.

For BPG and BPM the initial recoveries were low (20 % and 47 %, respectively) but this was due to the presence of salt in the seawater which reduces the response when comparing with ultra-pure water (see Figure 5). In fact, these two compounds were the ones disadvantaged for the addition of NaCl as we could see in the experimental design optimization (see section 3.3). To get good recoveries for this type of water calibration standards containing NaCl (15 %) were prepared. The recoveries obtained were favourable with values of 73 % and 94 % for BPG and BPM, respectively (see Table 6).

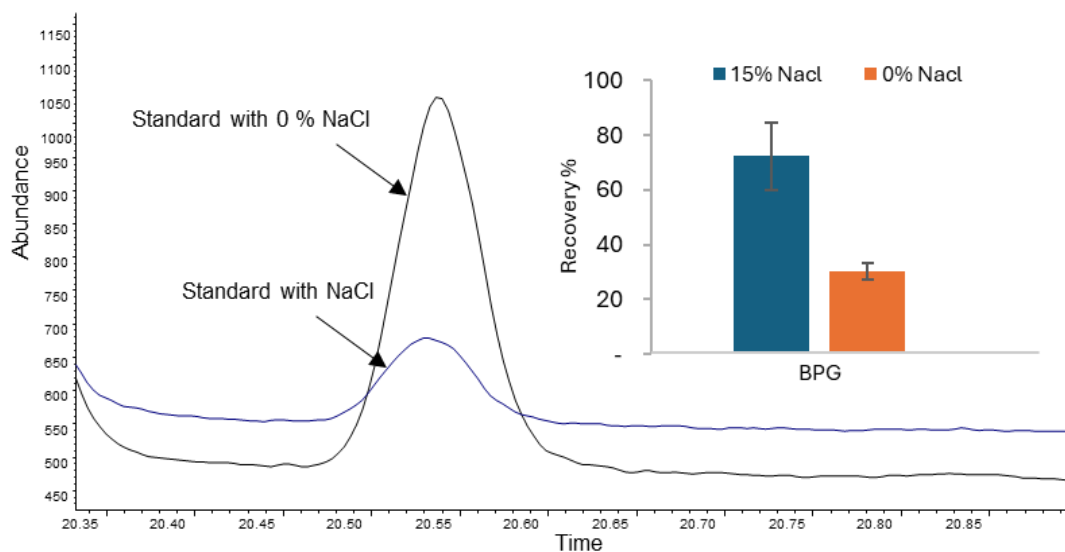


Figure 5. Ion chromatogram (m/z : 297) and responses obtained for BPG at 0 % and 15 % NaCl in standard water solutions. The recoveries in both cases are also compared.

Mean recoveries are given in Table 5 and, as can be seen they were generally higher than 90 %. The precision in real samples was also suitable for all the samples, and the mean precision values between 6 – 12 % considering all the real samples (seawater, river water, wastewater, and spring water) are also shown.

Table 6. Average recoveries in various environmental and wastewater samples.				
Compounds	Recoveries in water matrices (%)			
	Spring water	River water	Wastewater	Seawater
BPAF	96.8	112	107	103
BPF	100	99.4	77.4	81.1
BPE	100	103	81.3	85.9
BPA	101	106	n.c.	82.9
BPB	106	n.a.	n.a.	75.3
BPG	89.7	96.8	83.4	72.5
BPC2	101	130	119	99.8
BPZ	96.2	122	107	84.4
BPM	82.1	110	100	94.0
n.a. not available n.c. not calculated since the sample concentration was higher than the spike level				

3.3.5. Greenness and practicability of the proposed methodology

In recent years, considerable progress has been made in the development of extraction methodologies aligned with the principles of green analytical chemistry (GAC) and green sample preparation (GSP). These strategies aim to utilise safer materials, reagents, and solvents, minimise experimental steps, reduce waste generation and energy consumption, and enhance sample throughput. In 2022, the metric tool AGREEPrep (Wojnowski et al., 2022) was introduced to assess the environmental sustainability of sample preparation procedures. The sustainability evaluation of the developed SPME-GC-MS method for determining BPs in environmental water samples yielded a favourable greenness profile, with an AGREEPrep score of 0.62. This reflects a well-balanced approach that prioritise sustainability while maintaining high sensitivity and reliability.

The developed method is assessed according to ten criteria, as specified in Figure 6a: performed *in situ*, since integration sample preparation and chromatographic analysis is possible using the combiPAL autosampler device (criterion 1); the only hazardous component is acetic anhydride, used in minimal volume (50 µL) for analytes derivatization (criterion 2); a significant contributor to the method sustainability is the extensive reuse of SPME fibres (approx. 200 times),

substantially reducing the consumption of consumables and generation of solid waste (criterion 3); the method also produces minimal waste of 15 mL of water sample including 50 μ L of acetic anhydride, complying with waste minimisation principles (criterion 4 & 5); throughput is moderate, with two sample prepared per hour, indicating a balance between speed and thoroughness of preparation (criterion 6); the procedure consist of two discrete steps, extraction and thermal desorption, and employs a semi-automated system that minimizes manual intervention while maintaining flexibility (criterion 7); energy consumption is 137.5 Wh per sample due to the power consumption of the stirrer plate (criterion 8); GC-MS is employed as the analytical instrument (criterion 9) and finally, the procedure involves three hazards, including flammable, corrosive and health hazard although, due only to the use of acetic anhydride, but operators safety consideration were carefully addresses (criterion 10).

In addition to the AGREEPrep assessment, the Blue Applicability Grade Index (BAGI), presented in 2023, was also applied to assess practicability (Manousi et al., 2023), yielding a score of 67.5 (Figure 6b). This score indicates a strong balance between analytical performance and operational feasibility. The BAGI assessment highlights the method's capability for quantitative and confirmatory (criterion 1); multi-element analysis 9 compounds (criterion 2); using sophisticated instrumentation (GC-MS) (criterion 3); with simultaneous preparation of one sample and miniaturized extraction techniques (criterion 4 & 5); the throughput of 2 samples per hour (criterion 6); and the use of commercially available reagents (criterion 7); further support the method's applicability in routine laboratory settings. No pre-concentration is required (criterion 8); the semi-automated operation increases efficiency without compromising quality (criterion 9), and finally, water samples (15 mL) can be classified as environmentally friendly (criterion 10).

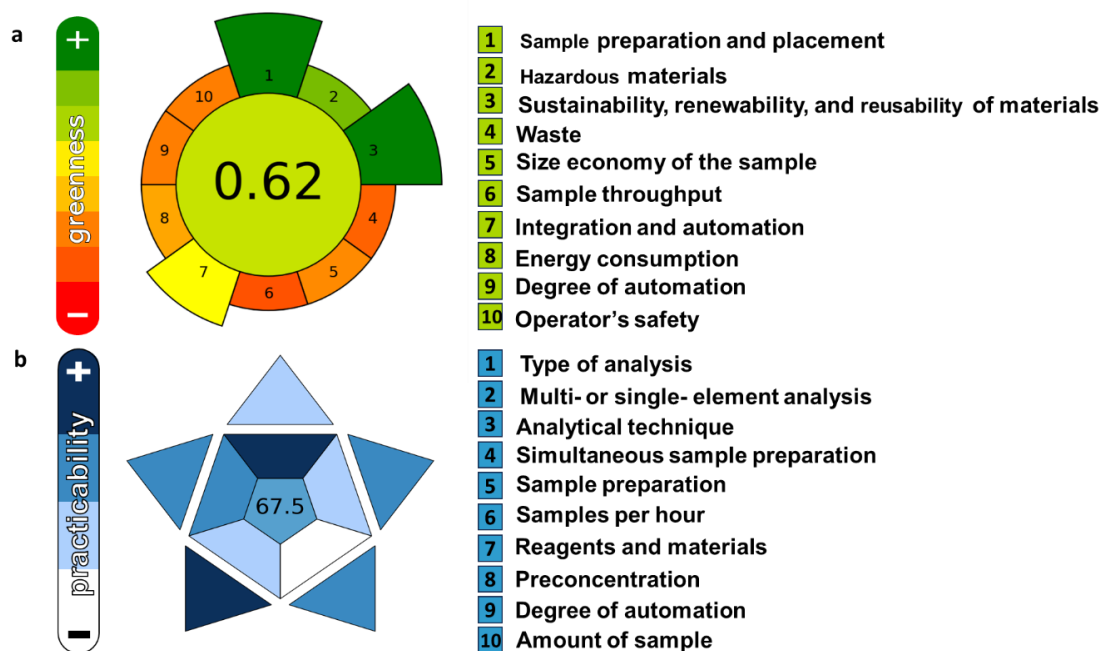


Figure 6. a) AGREEPrep greenness assessment of the optimized SPME-GC-MS method and, b) BAGI assessment of SPME-GC-MS as practicability of the method for BPs analysis in water matrices.

3.3.6. Comparison with other methods

Table 7 presents a comparison between the current SPME-GC-MS method and previous analytical methods reported for BPs in various environmental water matrices. The number of BPs analysed in this study (nine compounds) aligns well with or exceeds those in other studies. A major advantage of the present method is the low sample volume required (15 mL), which is considerably less than those employed in the most solid-phase extraction (SPE) methods, where volumes typically vary from 200 to 2000 mL. This reduction in sample volumes improves efficiency and reduces solvent consumption, aligning with green analytical chemistry principles.

The good linearity range ($0.005\text{--}5\ \mu\text{g L}^{-1}$) and low LOQ ($0.00044\text{--}0.0070\ \mu\text{g L}^{-1}$) achieved by the method demonstrate its high performance which is crucial for detecting BPs at trace levels in environmental waters. LOQs were comparable or superior to earlier methods that reports values between $0.01\ \text{ng L}^{-1}$ and several $\mu\text{g L}^{-1}$. Recovery rated for most BPs ranged between 91–107 % with RSD ranging from 6 % to 12 %, indicating reliable method accuracy across diverse water matrices such as seawater, river water, wastewater and tap water. These recoveries compare

favourably with those previously reported, which typically fall between 70 % and 120 % depending on the analyte and matrix complexity.

An important feature of this study is the application of *in-vial* acetylation derivatization, which enhances analyte volatility and chromatographic performance while simplifying sample preparation by integrating derivatization directly in the extraction vial. This approach reduces sample handling time and potential analyte losses and is consistent with green chemistry initiatives, offering advantages over separate derivatization/extraction steps in other methods. In brief, the proposed SPME method exhibits satisfactory performance and offers several advantages over conventional approaches.

Table 7. Comparison of the proposed SPME-GC-MS method with other methods developed for the determination of BPs in water samples.

Water matrix	Analytes	Method	Sample volume (mL)	Linearity	LOQ	Recovery (%)	Reference
Source water and drinking water	BPA, BPAF, BPAP, BPB, BPBP, BPC, BPC2, BPE, BPF, BPG, BPM, BPP, BPPH, BPS, BPTMC, BPZ	SPE-UPLC-MS/MS	500	0.05-50 $\mu\text{g L}^{-1}$	0.1-1.7 $\mu\text{g L}^{-1}$	73.1-96.9 Except BPPH (57.1)	(Česen et al., 2018)
Raw (influent) wastewater and treated (effluent) wastewater	BPF, BPE, BPA, BPC, BPB, BPG, BPC12, BPZ, BPAP	USAEME-GC/MS	5	0.005-500 $\mu\text{g L}^{-1}$	correspond to lowest calibration points (0.005 $\mu\text{g L}^{-1}$)	88-133 (ultrapure water), 85-119 (raw wastewater), 87-122 (treated wastewater)	(Kiejza et al., 2022)
Seawater (Surface and sediment)	BPA, BPAF, BPS, BPF, BPB, DHBP	SPE-UPLC-MS/MS	500	0.1-20 ng mL^{-1}	0.02-0.36 ng L^{-1} (in water), 0.01-0.12 ng g^{-1} dw (in sediment)	71-102 (in water), 78-97 (in sediment)	(Gao et al., 2023)
River and sediment	BPA, BPS, BPF, BPAF, BPAP, BPB, BPC, BPE, DHBP, TDP, TMBPA, TBBPA, TCBPA	LLE-UHPLC-MS/MS (pre-column derivatization)	10	0.005-5.0 ng mL^{-1} (water), 0.03-50 ng g^{-1} (sediment)	0.005-0.02 ng mL^{-1} (water), 0.80 ng g^{-1} (sediment)	81.1-119.1 (water), 80.9-111.5 (sediment)	(Wang et al., 2018)

Table 7. Comparison of the proposed SPME-GC-MS method with other methods developed for the determination of BPs in water samples.							
Water matrix	Analytes	Method	Sample volume (mL)	Linearity	LOQ	Recovery (%)	Reference
Tap water, purified water, bottled water	BPA, BPS, BPF, BPAP, BPP, BPAF, BPZ	SPE-LC-MSMS	250	0.01-50 ng mL ⁻¹	0.04-1.0 ng L ⁻¹	77-107	(Lim et al., 2023)
Coastal and estuarine surface water	BPA, BPF, BPS	SPE-LC-MS/MS	200	0.11-1.56 ng L ⁻¹	5-200 ng L ⁻¹	>90	(Zainuddin et al., 2023)
River and lake water	BPA, BPAF, BPAP, BPC, BPB, BPF, BPFL, BPS, BPZ	SPE-UPLC-MS/MS	500	0.5-200 ng L ⁻¹	0.011-0.077 ng L ⁻¹ (LOD)	80-121 (Milli Q water), 69-114 (spiked samples)	(Jin & Zhu, 2016)
Wastewater and river water	BPAP, BPAP, BPB, BPC, BPE, BPF, BPS, BPZ	SPE-GCMS (derivatization with MTBSTFA + TBDMCS)	2000	4-100 ng L ⁻¹	0.465-4.13 ng L ⁻¹	Except BPC (56.1)	(Zhang et al., 2019)

3.3.7. Application to real samples

To assess the applicability of the developed SPME-GC-MS method, it was applied to the analysis of real environmental water samples collected from a variety of sources. These included surface waters (rivers and seawater), recreational waters (swimming pools and football field irrigation), drinking water (tap, mineral, and ice cube water), and wastewaters (municipal and port discharges) given a total of 19 samples.

Results are shown in Table 8. Blank cells indicate concentrations below the LOD. Seven out of the nine target BP compounds were found in the analysed samples. BPA was the most frequently detected compound present in 13 of 19 samples, with concentrations ranging from 0.007 to 1.65 $\mu\text{g L}^{-1}$. This prevalence aligns with the previous studies that reported in the referenced review that BPA as a common environmental contaminant due to its extensive industrial use and persistence in aquatic systems (Corrales et al., 2015). BPF and BPE were detected in eight samples at concentrations between 0.005- 0.038 $\mu\text{g L}^{-1}$ and 0.001-3.48 $\mu\text{g L}^{-1}$ respectively. BPF showed the higher concentration in artificial turf crumb rubber football field irrigation water, while BPE in mineral water collected from the bottom of the water can of drinking water system, possibly reflecting localized contamination sources. BPAF was also found in five samples at concentration from 0.001- 0.006 $\mu\text{g L}^{-1}$, showing the highest concentration in wastewater. BPB was detected in three samples, with concentration up to 0.01 $\mu\text{g L}^{-1}$. BPG and BPC2 were each detected in one sample at concentrations approximately 0.01 $\mu\text{g L}^{-1}$. BPZ and BPM were not detected in any of the samples. Tap water (TW2) and ice cube water (ICW) from the same water source showed comparable results except the higher concentration of BPA in ICW, which could be due to the leach from the ice cube tray.

Table 8. Concentration of the target BP compounds in different types of water samples ($\mu\text{g L}^{-1}$).

	BPAF	BPF	BPE	BPA	BPB	BPG	BPC2	BPZ	BPM
MQ									
SPW1		0.011	<i>0.003</i>	0.084					
SPW 2									
SPW 3	<i>0.001</i>	0.011	3.48	0.021	0.005				
WW1	0.006	0.035	0.006	0.14					
RW				<i>0.007</i>		0.010			
TW1				0.013					
TW2		0.006	<i>0.001</i>						
ICW		0.005	<i>0.003</i>	0.038					
SP1	<i>0.001</i>								
SP2	<i>0.003</i>	0.018	<i>0.001</i>	0.19	<i>0.003</i>				
SW1									
SW2				0.025			0.009		
SW3									
FW1		0.030	<i>0.003</i>	1.65					
FW2	<i>0.003</i>	0.038	0.005	0.40	0.01				
PW1		LOQ		0.32					
PW2				0.23					
PW3				0.43					

MQ: Mili Q water; SPW: Spring water; WW: Wastewater; RW: River water; TW: Tap water; ICW: Ice cube water; SP: Swimming pool; SW: Seawater; FW: Football filed irrigation water; PW: Port water, Values in italics are semi quantitative, since they are below the calibration range.

Overall, these findings demonstrate the method's high sensitivity and suitability for simultaneous determination of multiple bisphenols in complex environmental water samples. The observed concentration ranges provide valuable insights into the distribution of bisphenol contamination in diverse water sources, underscoring the method's potential for routine analytical use, environmental monitoring and risk assessment programs.

3.4. Conclusion

An SPME-GC-MS method was developed and validated for the concurrent determination of nine BP compounds in drinking water, wastewater and environmental water samples. A low-cost *in-vial* derivatization was implemented to enhance the chromatographic response of the BPs. Direct *in-vial* acetylation using inexpensive reagents (NaHCO_3 and acetic anhydride) minimised analyte losses, prevented contamination, and enabled efficient derivatization within a short timeframe. The key factors effecting the SPME procedure were optimised through a design of experiments strategy, leading to the identification of optimal operating conditions. The final optimised method employed a DVB/CAR/PDMS fibre under DI extraction conditions at 100 °C for 30 minutes, with the addition of NaCl.

After optimising BP derivatization with SPME-GC-MS, the method was validated, showing excellent linearity, repeatability, and reproducibility. Recovery experiments across various water matrices confirmed quantitative recoveries, confirming the accuracy of the method.

Using GC-MS in SIM mode, LODs in the sub- and low $\mu\text{g L}^{-1}$ concentration range were achieved, which is crucial for detecting these emerging pollutants in water samples. Lastly, nineteen real water samples from different origins were analysed and 7 out of the 9 target BPs (BPAF, BPF, BPA, BPB, BPG, and BPC2) were detected. BPA was the most frequently found; it was present in fourteen of the samples.

In summary, the proposed methodology demonstrates both its suitability and high sample throughput for the determination of bisphenols in water samples. Key advantages of the optimised method include its low cost and minimal environmental impact, attributable to the use of only microliter quantities of organic solvents and the generation of the negligible waste, primarily consisting of the water samples themselves. This methodology thus represents a sustainable approach aligned with the principles of green chemistry.

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4. Pyrolysis-GC-MS Analysis of Microplastics in an *In Vitro* Colon Model

Extending the sludge-based analytical approach to the human gastrointestinal realm. Building on the methodologies validated in sewage sludge, we applied the same preparation and detection techniques to an *in vitro* colon fermentation model. This strategy was predicted on the insight that both sewage sludge and gut media present organic rich environment that hinder MPs detection, meaning the clean-up and identification methods successful in sludge should likewise prove effective in the colon system. Accordingly, the colon model study implemented an oxidative digestion of the fermentation matrix (adapting the Fenton's reagent protocol from the sludge study) to eliminate organic debris, coupled with Py-GC-MS for sensitive polymer identification and quantification. While the context differ, one environmental and one biomedical, the analytical complexity is a common barrier to accurate MPs quantification in both cases.

4.1. Introduction

In recent decades, the pollution caused by Microplastics (MPs), which include both primary types (such as microbeads and pellets deliberately created) and secondary types (fragment that result from the breakdown of larger plastics), has escalated significantly. The rise is due to the increase in production, wide spread use, inefficient disposal, and the very slow natural breakdown of plastics in the environment (Napper et al., 2015; Prata, 2018). MPs have been detected in various environments including surface water (Carbery et al., 2018; Eriksen et al., 2013; La Daana et al., 2017), beaches (Herrera et al., 2018; Imhof et al., 2018), seabed sediments (Browne et al., 2011; Karlsson et al., 2017; Rodrigues et al., 2018), wastewater effluents (Murphy et al., 2016; Ziajromi et al., 2016), and fresh bodies (Horton et al., 2017). They have been found in sea-ice in both the Arctic and Antarctic (Waller et al., 2017) and in various living organisms (Li et al., 2016; Neves et al., 2015). Moreover, MPs have been identified in products like salt and honey (Karami et al., 2017; Liebezeit & Biebezeit, 2013), bottled water (Oßmann et al., 2018), as well as in both indoor and outdoor air (Dris et al., 2017; Gasperi et al., 2018; Prata, 2018; Vianello et al., 2019).

Microplastics represents a significant environmental issue, affecting various aspects of global ecosystems. Despite their widespread presence, particularly across diverse life forms and environmental settings, the understanding of their potential health effects on humans remains limited (Rahman et al., 2021). MPs and Nanoplastics (NPs) could potentially presents a variety of

health concern for humans, including metabolic changes, immune disruptions, and other physiological impacts. Research has shown that exposure to MPs can lead to alteration in immune system, increases neurotoxic effects, reproductive toxicity, carcinogenicity, and various cellular stress responses such as oxidative stress and inflammation (Cf et al., 2021; Rahman et al., 2021; Wu et al., 2022; Yong et al., 2020). Different plastic types, such as polyethylene terephthalate (PET), may have distinct health impacts, with some plastics known to act as endocrine disruptors or lead to reproductive issues, and polyethylene seems to be leading to reproductive difficulties in mice (Park et al., 2020; Sax, 2010)

Recent studies found that MP existed in the human colon, lung and placenta, and might influence human health (Amato-Lourenço et al., 2021; Ibrahim et al., 2021; Ragusa et al., 2021). MPs may alter the gut microbiome of various species, including mussels, zebrafish, shrimp, and murine models, and this has raised concern about potential risks to human health (Chae et al., 2019; Jin et al., 2019; Li et al., 2020; Lu et al., 2018; Qiao et al., 2019; Su et al., 2024). The gut microbiome is a complex community of bacteria, archaea, fungi, protozoa, and viruses, playing a vital role in human health by regulating metabolism, immune responses, and the overall health of the host (Gałęcka et al., 2025).

Artificial or simulated digestion models provided first insights into the impact of micro- and nanoplastics (MNPs) on human gut microbiota. Static digestion of MPs revealed that polyethylene PE particles, particularly in the size range of 30-140 µm, caused an increase in the abundance of bacteria such as *Bacteroides*, *Clostridium*, *Escherichia* in the gut microbiota. This effect was more pronounced when the plastic additive tetrabromobiphenol A was present (Huang et al., 2021). A standardized *in vitro* model using a dynamic gastrointestinal system also showed that PET particles underwent biotransformation during digestion, leading to alteration in the gut microbiota. The transformation resulted in an increase in bacteria like *Escherichia/Shigella* and *Bifidobacteria*, and other opportunistic pathogens such as *Phascolarctobacterium*, *Lachnoclostridium*, and *Megasphaera*. Additionally, microbial binding in a multi-compartment model promoted biofilm formation (Tamargo et al., 2022). Repeated exposure to PE in the Mucosal Artificial Colon model led to an increase in *Desulfovibrionaceae* and *Enterobacteriaceae*, while reducing *Christensenellaceae* and *Akkermansiaceae* in adult stool samples. In contrast, infant stool showed an increase in the alpha diversity of *Desulfovibrionaceae* and *Enterobacteriaceae* (Fournier, Leveque, et al., 2023;

Fournier, Ratel, et al., 2023). In the mucosal simulator of the human intestinal ecosystem, PET particles affected both the intestinal luminal and mucosal microbiota, with more pronounced effects observed in the lumen due to the stabilizing effect of mucus. Gut microbes such as *Acidaminococcus* and *Morganella* were found to release phthalates from MPs, which significantly affected luminal and mucosal microbiota (Yan et al., 2022). Furthermore, the impact of MPs on the gut microbiome could potentially influence the bioavailability and toxicity of other environmental contaminants, such as pesticides and heavy metals, by altering the gut's detoxification and absorption processes (Chiu et al., 2020; Hernández-Mendoza et al., 2022; Tang et al., 2024).

Most exposure studies in this area (i) chemically confirms particles in clinical tissues using μ Raman/ μ FTIR (e.g., lung, colon, placenta (Amato-Lourenço et al., 2021; Ibrahim et al., 2021; Ragusa et al., 2021) or (ii) report only the nominal MP dose added at the start of the exposure focusing on biological outcomes, gut microbiota, or toxicity, without re-identifying or re-quantifying the MPs during the experiment (Chae et al., 2019; Chiu et al., 2020; Fournier, Leveque, et al., 2023; Gałęcka et al., 2025; Jin et al., 2019; Li et al., 2020; Lu et al., 2018; Qiao et al., 2019; Ragusa et al., 2021; Su et al., 2024; Tamargo et al., 2022). A notable gastrointestinal simulation example characterized PET particles across digestion using Raman (chemical structure) and electron microscopy (morphology/biofilm) (Tamargo et al., 2022), but did not provide polymer mass over time.

The present study employed a Py-GC-MS analytical workflow to qualitatively and quantitatively determine PE and PS MPs in samples from an *in vitro* colon model over the course of the fermentation process. Within an interdisciplinary collaboration, the specific objective was to monitor how controlled exposure to PE and PS MPs alter gut microbial community structure and metabolic activity. Building on this analytical and microbial characterization, the study sought to assess the potential health relevance of these shifts by linking microbiome dysbiosis and metabolic alterations to plausible disease pathways (Nissen et al., 2024).

Fenton oxidation was used as sample pre-treatment and quantification by internal standard normalization using 1,3,5-tri-*tert*-butylbenzene added directly to the pyrolysis cup as a practical surrogate in the absence of deuterated polymer standards leveraging its combines aromatic-aliphatic character common to the PE/PS mixture utilized in the experiments.

4.2. Material and Methods

4.2.1. Polymers and chemicals

All chemicals and reagents used in this study were of analytical grade. 1,3,5-tri-*tert*-butylbenzene, hydrogen peroxide solution (30% w/w), Iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), ethanol (99.0%), and toluene were purchased from Sigma-Aldrich. A certified microplastics standard consisting of polyethylene embedded in silica (SiO_2) matrix (Frontier Lab PY1-4940, SiO_2MP) was used for calibration and method validation; this material 150.7 μg of polyethylene in 4 mg of solid mixture. A polystyrene (PS) standard was obtained from Frontier Lab, and prepared as a stock solution in toluene at a concentration of 0.25 mg L^{-1} . All reagents, solvents, and polymer standard were handled under laboratory conditions to minimize contamination and ensure analytical reliability.

4.2.2. Quality control

All the chemicals and solvents used in the protocol were filtered through 0.3 μm quartz filters that were pretreated in a muffle furnace for 4 hours at 600 $^{\circ}\text{C}$, and all glassware was rinsed three times with filtered water and acetone prior to use and covered with aluminum foil to avoid external contamination. Airborne contamination (MPs) was evaluated, using the quartz filters identical to those used in analytical workflow were positioned in three locations: in the working hood area, adjacent to weighing balance, and near the pyrolyzer to passively collect the particulates (Figure 1). Following Hu et al, (Hu et al., 2019) the exposed filters were analyzed by Py-GC-MS under the same conditions as the samples. In particular, analogous 10 mm diameter quartz filters were prepared similar to those used for the treatment of the samples. These filters were placed on Petri dishes. Procedural and air blanks for air contamination were included at each set of analysis. Inspection of the pyrograms of the blank controls did not reveal the presence of pyrolysis markers of PP, PET, polyamides, poly(methyl methacrylate).

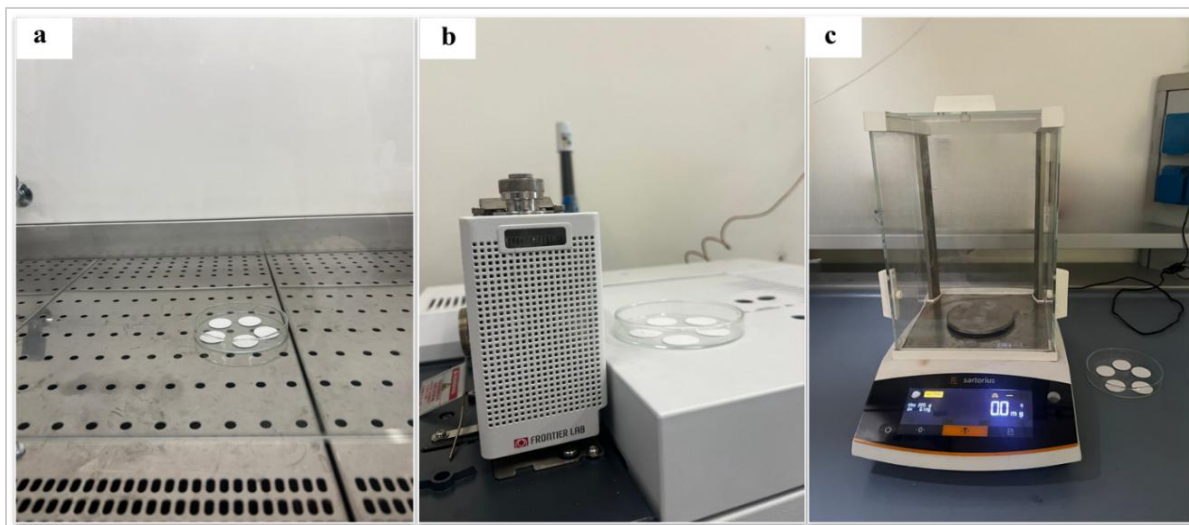


Figure 1: Arrangement of filters for air sampling in the workplace, (a) under the working hood, (b) near the pyrolyzer, and (c) next to the weight balance.

4.2.3. Experimental model of the human colon

These experiments were carried out by the research groups of the *Department of Agricultural and Food Sciences, Department of Biological, Geological and Environmental Science and the Centre for Applied Biomedical Research, of the University of Bologna* (Nissen et al., 2024). PE and PS MPs (PS microspheres ($\sim 3 \mu\text{m}$) from Polysciences (Warrington, PA, USA), while PE microspheres ($1\text{--}4 \mu\text{m}$) were purchased from Cospheric LLC, Somis, CA, USA) were subjected at 1:1 w/w load to standardized oro-gastro-duodenal digestion (INFOGEST), washed, vacuum dried, and introduced into a multi-unit *in vitro* colon fermentation (MICODE) inoculated with pooled human fecal microbiota from three healthy adults under strictly anaerobic, 37°C , pH controlled condition for 48 hours (Figure 2). Three parallel MICODE vessels (being 90 mL the volume of the bioreactor) were run: control (water) and two MP loads derived from single-meal exposure estimates 0.166 g (higher dose) and 0.033 g (lower dose). The working amounts chosen for this work were derived from the mass-based data reported by Senathirajah et al. (Senathirajah et al., 2021). Three points were considered for each vessel, baseline (after adaptation of fecal inoculum, 24 hours and 48 hours). Aliquots of the batch cultures (in the 3 – 10 mL range) were stored at -20°C prior to analysis (Py-GC-MS after Fenton oxidation and filtration).

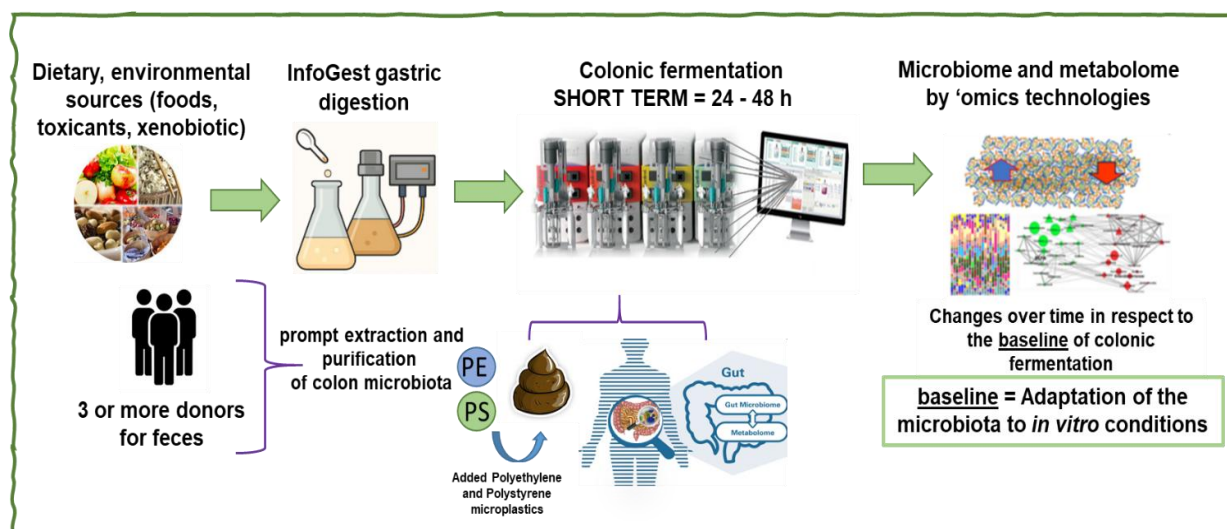


Figure 2: Workflow of In vitro simulation of intestinal digestion by MICODE gut model.

4.2.4. Fenton oxidation

Aliquots from bench culture were quantitatively transferred into pre-cleaned glass Erlenmeyer flasks that had been dried at 90 °C and pre-weighed. The resulting solid residues (ranging from 40 to 490 mg) were subsequently subjected to Fenton oxidation to minimize organic matrix interference. Fresh acetic acid/acetate buffer solution was prepared by diluting 7.5 mL of 1 M acetic acid to 100 mL with filtered water in a volumetric flask. In a separate vessel, 0.82 g of sodium acetate was dissolved in 10 mL of filtered water. These two solutions were then combined at 9:1 (v/v) ratio to generate the working buffer. A ferrous sulphate solution in this buffer was freshly prepared by weighing 2.78 g of $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ in the acetic acid/acetate buffer and transferrin quantitatively into a 100 mL volumetric flask. The solution was diluted to the mark and filtered through a glass fiber filter under vacuum. Due to the instability of ferrous ions in solution, this reagent was prepared immediately prior to use.

For the oxidation procedure, dried samples were transferred into a 100 mL Erlenmeyer flasks containing 25 mL of filtered water. The volumes of FeSO_4 buffer and hydrogen peroxide (H_2O_2 , 30% w/w) were calculated relative to the total water volume. The FeSO_4 buffer was added at a volume ratio of 4.2:1 (sample water : buffer), corresponding to 5.9 mL of buffer per 25 mL of water. Filtered H_2O_2 was introduced at a volume ratio of 1.6:1 (H_2O_2 : water), totaling 15.6 mL per 25 mL water, administered in two equal aliquots. Initially, half of the H_2O_2 volume (7.8 mL) was added, and the flasks were placed in an ultrasonic bath operating in altering cycles of 10 min

sonication and 10 min rest for a total duration of 1 hour. Subsequently, the remaining H₂O₂ (7.8 mL) was added, and the ultrasonic treatment was continued for an additional hour following the same cycle protocol.

Following oxidation, the mixture was filtered through quartz fiber filters (Munktell, 0.3 µm pore size, 25 mm diameter) that had been pre-conditioned in a muffle furnace at 600 °C for 4 hours. Filters were sequentially rinsed with distilled water and 30 % (v/v) ethanol to remove residual reagent, then dried at 90 °C until constant mass. Each filter was weighed gravimetrically, and a weighed portion was transferred into a pyrolysis cup. Prior to analysis, 5 µL of internal standard solution (TTB, 100 mg L⁻¹ in toluene) was added to the sample for quantification purposes, followed by Py-GC-MS analysis.

4.2.5. Quantitation

Standard mixture of polymers have been characterized for the identification of the peaks attributable to the molecules marker of each polymer. Thus, by means of the mass spectra, it was possible to identify the compounds corresponding to each peak, subsequently assigned to each polymer through comparison with data from previous studies or literature. In Table 1 the GC-MS data of pyrolysis markers selected to quantify PE and PS.

Table 1: List of the pyrolysis markers of PS and PE, retention time and m/z of ions in the mass spectrum (in bold the ion used for quantitation).			
Polymer	Marker	Retention time min	ions m/z
PE	C14-C20 <i>n</i> -alka,α,ω-diene	from 16 to 26	41, 55, 82 , 96
PS	styrene trimer (2,4,6-triphenyl-1-hexene)	40.1	91, 117, 194, 207, 312

For calibration, the mass of each polymer was correlated to the area of one (or more) specific markers; therefore, the integration of the areas under the peak of interest for each marker was carried out. For each analysis, the Total ion chromatogram (TIC) was recorded and in order to reduce baseline noise on the peak to be integrated, the integration was performed on the Extracted

ion chromatogram (EIC) at m/z 82 for alkadienes and m/z 312 for styrene trimer, thus obtaining a new chromatogram corresponding to the target ion of each marker.

Calibration curves constructed for PE from the Frontier-Lab standard of MPs in solid mixture with silica (SiO_2) by exact weighting different amounts of the mixture up to 4.09 mg (i.e. 151 μg of PE) (Figure 3). In the case of PS, different weighted volumes of the standard toluene solution (0.25 mg L^{-1}) were pyrolyzed PS a neat Frontier-Lab PS standard using the internal standard method (Figure 4). The relationship between the IS normalized EIC peak area and the IS normalized mass of the pyrolyzed polymer was expressed by the following linear equation:

$$\frac{A_x}{A_{IS}} = a + b \frac{\mu g_x}{\mu g_{is}}$$

Where A_x is the peak area of the investigated polymer marker integrated at its characteristic m/z , A_{is} is the peak area of the internal standard TTB integrated at m/z 231, and μg_x is the pyrolyzed mass (μg) of the polymer in each run and μg_{is} the mass of spiked IS. The coefficients ***a*** and ***b*** represents the intercept and slope of the calibration line, respectively. The lowest concentration value in the calibration curves corresponded with the LOQ, 0.5 μg for PS and 4.5 μg for PE.

For each polymer, increasing masses of standard material were pyrolyzed under identical conditions while maintaining a constant amount of the internal standard. The resulting ratios A_x/A_{is} were plotted as a function of the corresponding polymer mass (W_x/W_{is}) to generate calibration curves.

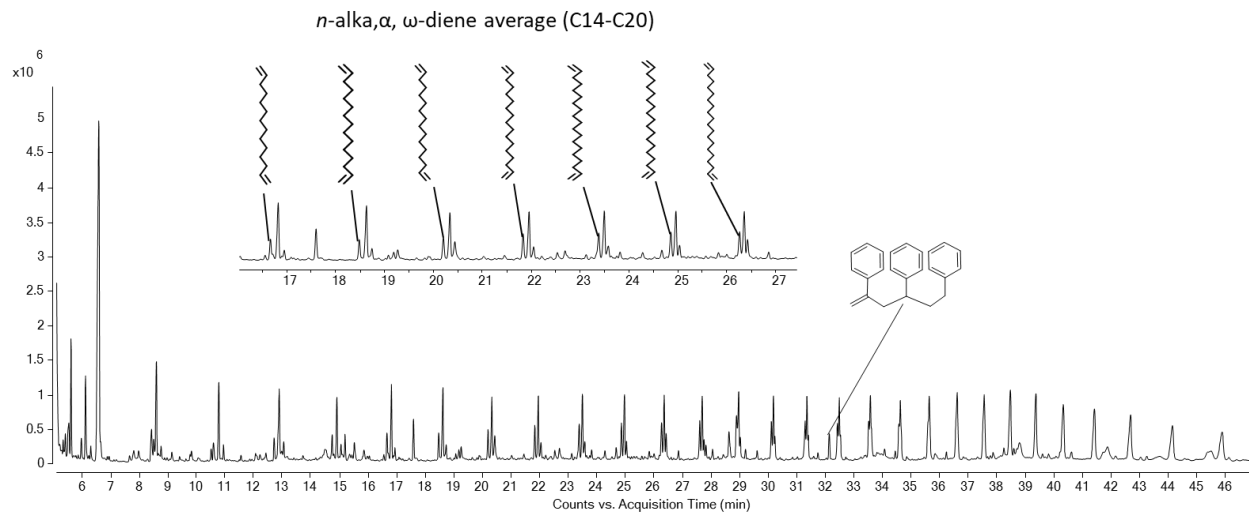


Figure 3: TIC pyrogram from pyrolysis of the MP mixture (4.09 mg) SiO₂ (frontier lab) in the presence of TTB internal standard.

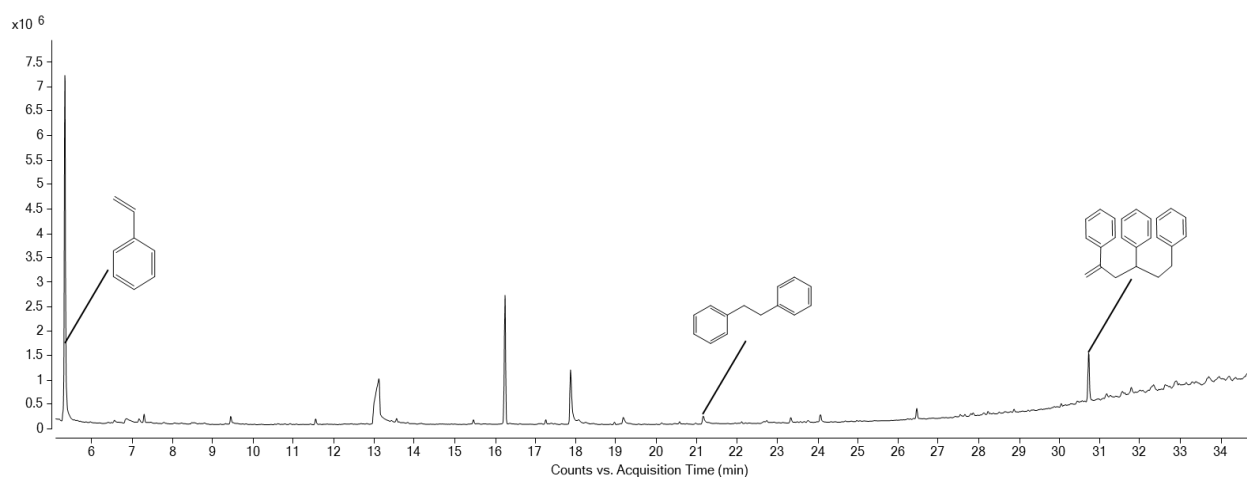


Figure 4: TIC pyrogram from pyrolysis of PS a neat Frontier-Lab standard in toluene solution (0.25 mg L⁻¹) in the presence of TTB internal standard.

4.3. Results and Discussion

In this study dried samples from multi-unit *in vitro* colon fermentation (MICODE) vessels at three conditions: water (control), added with 0.033 g and 0.166 g PE+PS 1:1 MP and three time points (baseline BL, 24 and 48 hours) were subjected to Fenton oxidation to reduce the organic matrix in accordance with other studies on similar matrices (Yan et al., 2021). Procedural blanks and contamination tests (section 3.2) were also performed between analyses. MPs contained in the sampled culture media and trapped onto the membrane filters were analysed by Py-GC-MS to

confirm the presence of MPs and evaluate actual concentrations during the duration of the fermentation experiments. In the resulting pyrograms of control samples, peaks associated to the organic matrix were clearly visible and as Figure 3 shows, consisting principally of volatilization or thermal degradation products of lipids (fatty acids, *n*-alkanes, steroids). Palmitic (16:0) and stearic acid (18:0) were the most abundant fatty acids, while the presence of odd-numbered fatty acids (15:0 and 17:0) was consistent with bacterial origin (Figure 5). This confirmed that the Fenton treatment reduced, but did not completely remove, the organic matrix. However, in control samples, the diagnostic pyrolysis markers of the targeted polymers were absent or appeared only as trace signals, whereas they were clearly detectable in the treated samples spiked with MPs, as shown in Figure 6 overlapped pyrograms of spiked and control samples.

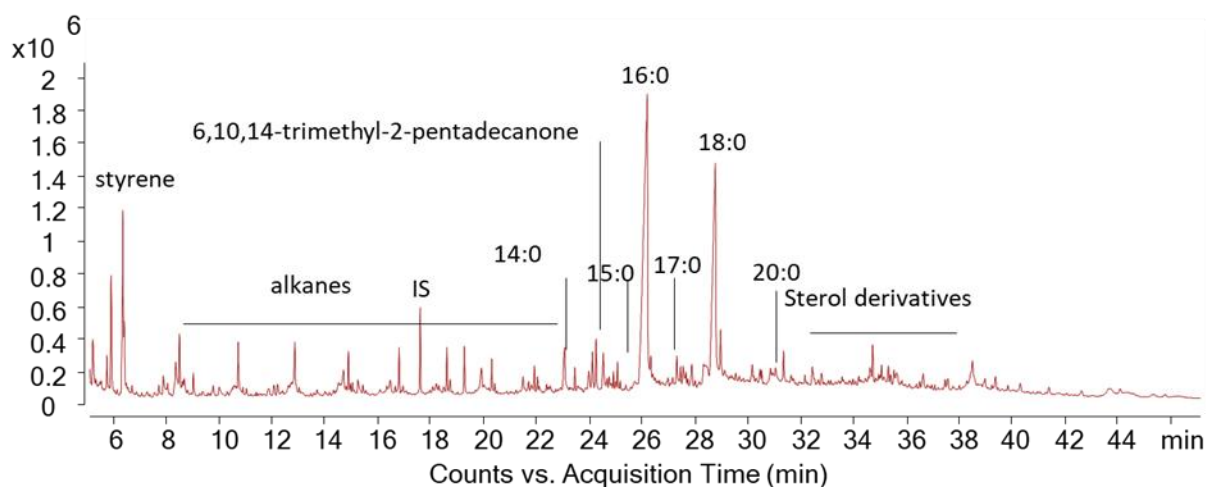


Figure 5: Pyrogram of MICODE effluent (Control, 24 h), highlighting matrix-derived lipid and sterol degradation peaks. Saturated fatty acids from 14:0 to 20:0.

Styrene monomer was abundant in control samples, therefore it was not a reliable pyrolytic marker of PS. Instead, the styrene trimer was negligible confirming a pyrolytic marker specific of PS. Normal alkanes and alk-1-ens are abundant pyrolysis products of PE, but prone to organic matrix interference especially from lipids. Multiple method evaluations have shown that fats and other organic matter generates the same alkane/alkene series as PE, causing positive bias unless the matrix is stringently removed or alternative markers are used (See section I. (4.4.3.2.)). Accordingly, *n*-alka, α , ω -diene were selected as primary quantifiers for PE which are less intense, but more specific than alkanes and alkenes.

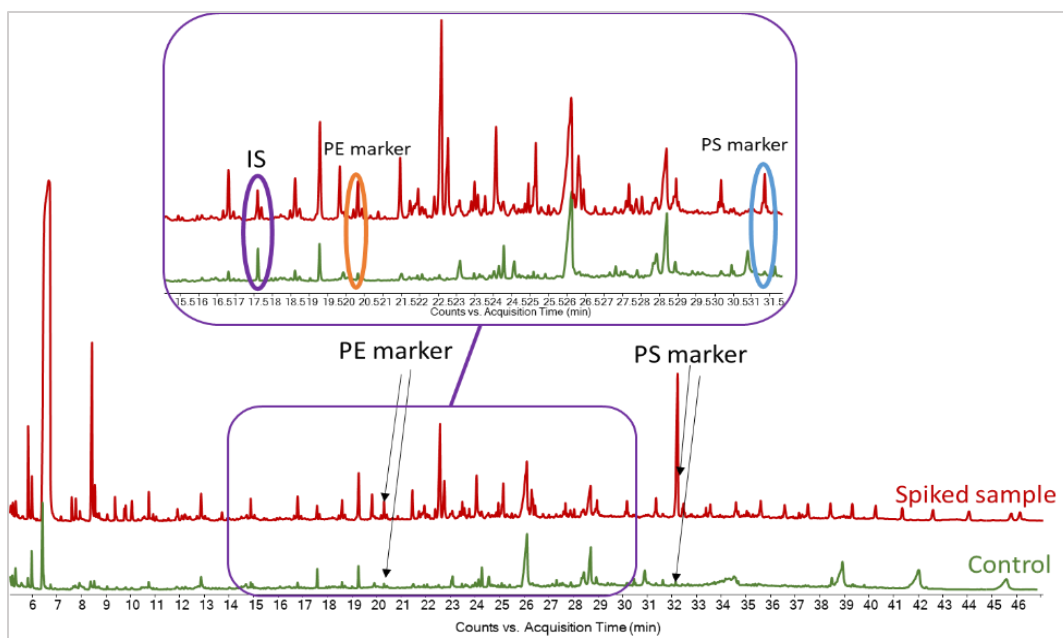


Figure 6: Overlaid TICs after Fenton; residual matrix in control; PE and PS evident in spiked samples.

All the pyrograms of treated samples exhibited the typical fingerprinting of PE (characterized by triplets of *n*-alka, α,ω -diene, *n*-alk-1-ene, *n*-alkane) and PS (characterized by styrene monomer, dimer and trimer) attesting the presence of the MPs throughout the experiment, as shown in the Figure 7 and 8.

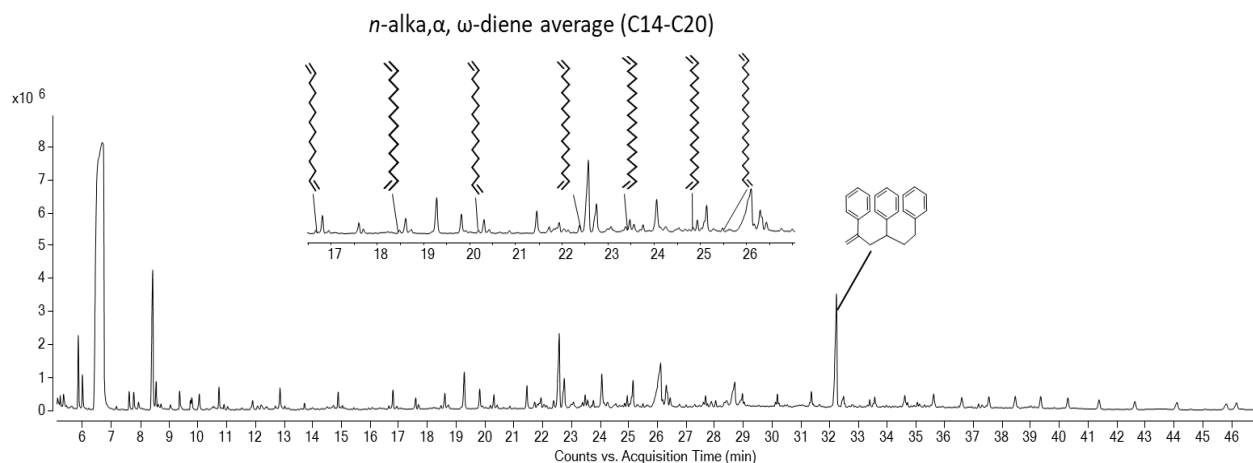


Figure 7. Fenton-treated total ion pyrogram of higher dose batch (0.166 g, 48 h) from Py-GC-MS showing PE and PS markers used for quantitation (m/z 82 and 312, respectively).

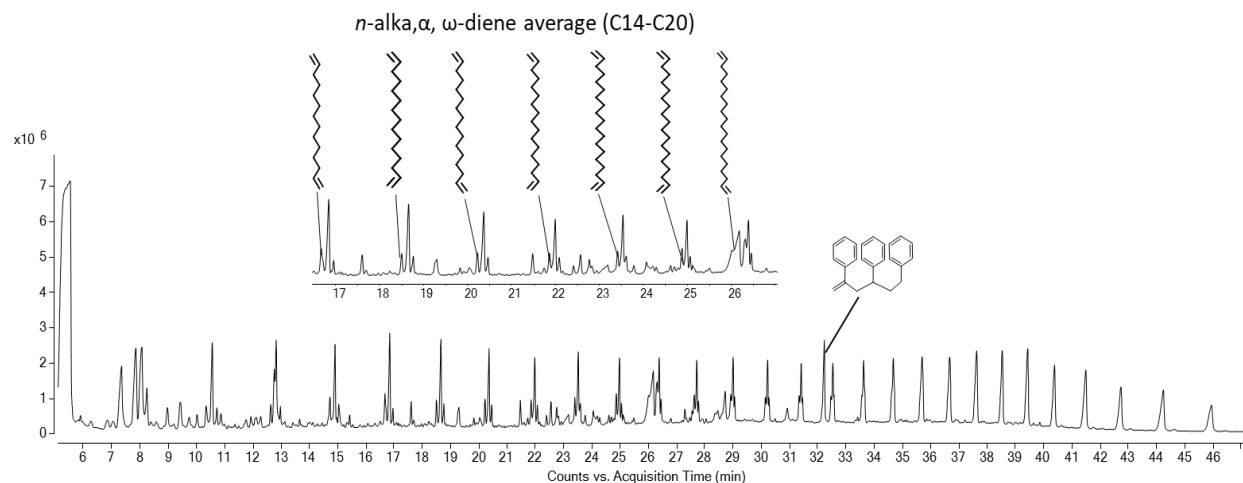


Figure 8. Fenton-treated total ion pyrogram of lower dose batch (0.033 g, 48 h) from Py-GC-MS showing PE and PS markers used for quantitation (m/z 82 and 312, respectively).

Polymer quantification was accomplished by the internal standard method with calibration curves constructed for PE and PS. PE was quantified using C_{14} - C_{20} n -alka- α , ω -dienes (quantification ion m/z 82) and PS using styrene trimer (quantification ion m/z 312). Calibration employed the internal standard method with multi-point curve showed a good linearity PE 4.5-154 μg ($n=7$, $R^2=0.983$) Figure 9, and PS 0.5-194 μg ($n=11$, $R^2=0.981$) Figure 10, prepared from commercial reference material (Frontier Lab). Reproducibility of IS peak area RSD 30 % for PS calibration ($n=11$) and 8 % for PE calibration suggesting the satisfactory behaviour of tri-*tert*-butylbenzene as internal standard (See Section I (4.4.3.4.)).

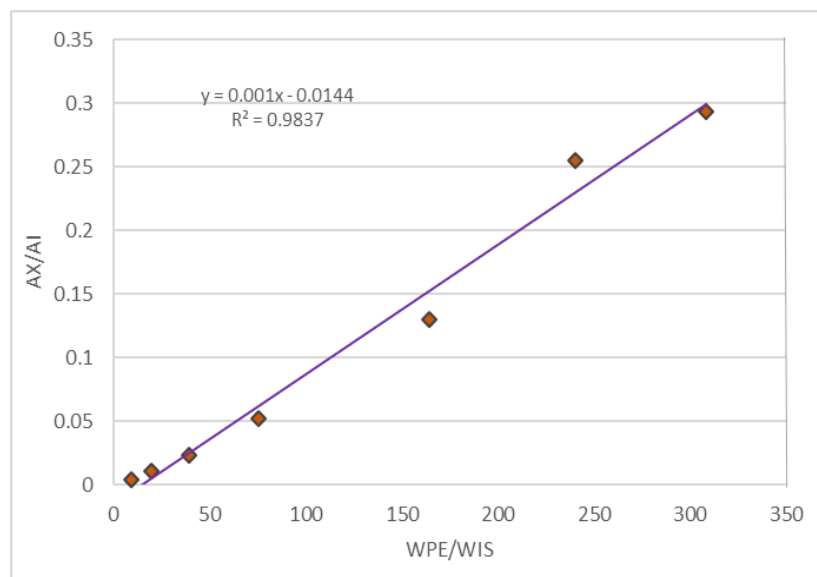


Figure 9: Calibration curve for PE (C_{14} - C_{20} n alka- α , ω -dienes; quantification ion m/z 82). $Y = A_x/A_i$, where A_x is the peak are of PE markers, and A_i is the peak area of internal standard TTB. $X = W_{pe}/W_{is}$, where W_{pe} is the mass (μg) of PE pyrolyzed in the cup and W_{is} is the mass (μg) of TTB added to the same cup.

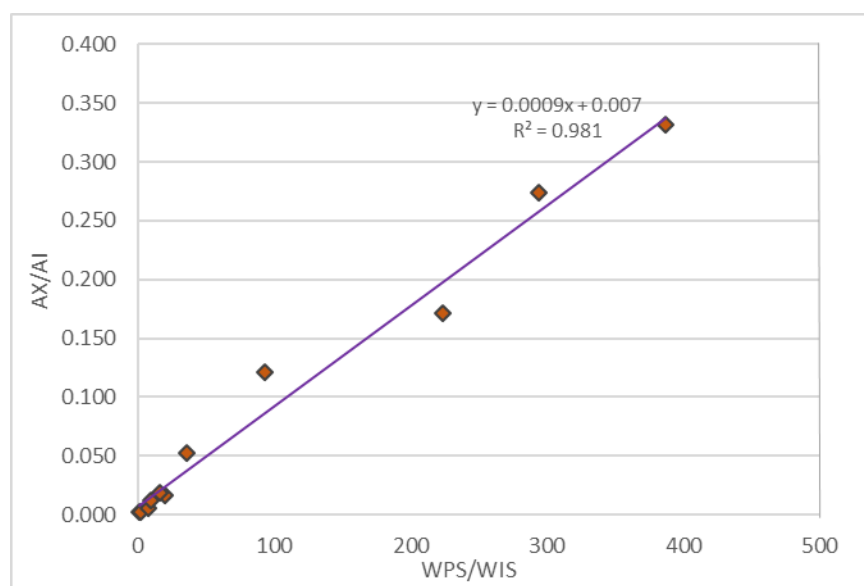


Figure 10: Calibration curve for the PS using styrene trimer (quantification ion m/z 312). $Y = A_x/A_i$, where A_x is the styrene-trimer peak area, and A_i is the peak area of internal standard TTB. $X = W_{ps}/W_{is}$, where W_{ps} is the mass (μg) of PS pyrolyzed in the cup and W_{is} is the mass (μg) of TTB added to the same cup.

In the MICODE colon fermentation, Py-GC-MS confirmed dose-dependent recovery of the spiked MPs: in the high-dose vessel (0.166 g total polymer), PS was quantified at $3.8 \pm 1.9 \text{ mg g}^{-1}$, and PE at $1.4 \pm 0.7 \text{ mg g}^{-1}$ dry weight (total 5.2 mg g^{-1}). In the low-dose vessel (0.033 g) PS was 0.7 ± 0.5 and PE was 0.5 ± 0.1 (total 1.2 mg g^{-1}); the water control remained <LOQ (Table 2). The quantitation data showed the difference of the measured PS:PE ratio from the nominal 1:1 value could be caused by the differential segregation of particles, for instance adhesion to labware during analytical sample pre-treatment (Kosuth et al., 2023), or possibly by PE partial degradation by human gut bacteria, as reported in a previous study by Jang et al. (Jang et al., 2024). Data presented a high variability probably due to the complexity of the matrix that effect sampling, sample preparation, and pre-treatment.

Table 2. Concentration (mg g^{-1} dry weight) of PE and PS MPs in MICODE vessels (mean values $\pm$ SD, averaged over the duration of the experiment).			
Sample	PS	PE	PS +PE
higher dose	3.8 ± 1.9	1.4 ± 0.7	5.2
lower dose	0.7 ± 0.5	0.5 ± 0.1	1.2
Control	< LOQ	< LOQ	< LOQ

In the absence of data on similar studies, a comparison could be made with concentrations determined by analytical pyrolysis in studies dealing with human feces. Although derived from a controlled *in vitro* exposure, concentrations were higher than those typically quantified in human feces by Py-GC-MS under real world exposure (e.g., in the $\mu\text{g g}^{-1}$ dw range). For instance, in a study of young adults, Py-GC-MS detected PE, PVC, PS, PP, PET (and often PA66) in 98.7% of participants, with a median total MP mass of $54.7 \mu\text{g g}^{-1}$ that are approximately 95-fold higher burden than our results (Song et al., 2025). Among pre-school children, Py-GC-MS quantified 11 polymers and reported an average total MP burden $\sim 338 \mu\text{g g}^{-1}$ dw, with frequent detection of PVC, PET, PE, PA6; this cohort also linked diet/behavior with stool MPs loads (Ke et al., 2023). In neonatal meconium, a validated Py-GC-MS assay for eight target polymers, confirming detection in real samples with concentrations in the tens-to-hundreds $\mu\text{g g}^{-1}$ range (Li et al., 2025).

Complementary analysis combining thermal-desorption GC-MS and infrared imaging revealed that small sized particles (0.2-20 μm) dominated human excretion, with the fine fraction averaging approximately $345 \mu\text{g g}^{-1} \text{dw}$ (Zhu et al., 2024). This divergence reflects the intentionally elevated, short-term exposure design used *in vitro* systems to elicit measurable microbial and metabolic responses.

In vitro exposure to the PE/PS mix induced a dose and time dependent dysbiotic shift: opportunistic bacteria overgrowth (*Enterobacteriaceae*, *Desulfovibrio spp.*, *Clostridium group I* and *Atopobium Collinsella* group) and a contextual reduction on abundances of all the beneficial taxa analyzed, with the sole exception of *Lactobacillales*. Together, these microbiomes and volatilome shifts provide mechanistic evidence that PE/PS MPs promote colon ecosystem changes consistent with dysbiosis within 24-48 hours under controlled human-microbiota fermentation. The potential health implications of these microbial changes could be profound, suggesting that MPs may influence host physiology beyond the gastrointestinal tract.

4.4. Conclusions

In contrast to previous studies that focused on the physiological/biological effects of MPs in digestion models used spectroscopic techniques, our research directly confirms the presence of MPs throughout the experiment and quantifies them using Py-GC-MS, providing polymer-specific mass measurements ($\mu\text{g-mg}$) rather than relying solely on initial particle counts or estimates at “time zero”. This approach is particularly suited to the organic rich colon model, which contains complex microbial biomass, proteins, lipids, polysaccharides, and metabolites, all of which can complicate analysis. Optical methods such as Raman or μFTIR may face limitations due to issues like auto-fluorescence, scattering, or surface fouling, whereas pyrolysis based methods can experience co-elution and matrix derived fragments that obscure polymer markers. To address these challenges, our workflow incorporates oxidative clean-up (e.g. Fenton oxidation), normalization with internal standards, supported by procedural blanks and recovery checks, to ensure reliable and accurate quantitative results for MPs in this complex system.

However, despite these advancements, factors such as sample pre-treatment, sample size, and interference from the complex organic matrix remain crucial considerations for achieving accurate and reliable results in analytical pyrolysis. In addition, the need for effective calibration methods such as matrix-matched calibration with proper MP standard, and the continued development of

standardized protocols for handling diverse environmental matrices are essential to further enhance the precision and applicability of Py-GC-MS in MPs quantification. These aspects, along with improvements in sampling techniques, will be key in reducing matrix effects and ensuring reproducible results across varied sample types.

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IV. Conclusion

This doctoral thesis presents the development and optimization analytical methods for the detection and quantification of three major classes of emerging contaminants including UV filters, bisphenols, microplastics in complex environmental water matrices and *in vitro* biological models. By integrating innovative sample preparation strategies with gas chromatography-mass spectrometry based techniques, this research addresses key challenges in environmental monitoring and contributes to improving the reliability and sensitivity of contaminants analysis in heterogeneous matrices. The research advances the field by employing integrated sample preparation and chromatographic techniques to address environmental monitoring challenges.

For the determination of UV filters in seawater, a solid-phase extraction (SPE) method coupled with gas chromatography-tandem mass spectrometry (GC-MS/MS) was optimized for the simultaneous determination of eleven UV filters and one transformation product. The method achieved limit of detection (LODs) below 1 ng L⁻¹ for most target compounds. Applying this method to fourteen seawater samples collected along Adriatic coast during peak recreational periods revealed the widespread presence of UV filters. Notably, octocrylene (74-23,100 ng L⁻¹), homosalate (17- 16,400 ng L⁻¹), and ethylhexyl salicylate (16- 10,900 ng L⁻¹) were among the most abundant, while trans-2-ethylhexyl methoxycinnamate (*E*-EHMC) was present at 13-1,4747 ng L⁻¹, with its photoisomer *Z*-EHMC, which accounting for 16-64% of the total. This study provides the first detailed dataset on UV filter contamination in the Adriatic Sea, thereby improving current understanding of environmental exposure and contributing to risk assessment.

Investigation of the photoisomerization behavior of *E*-EHMC demonstrated its rapid conversion to *Z*-EHMC under ultraviolet irradiation in aquatic matrices, cosmetic formulations, and an *in vitro* human skin model. Photo stationary states were reached within minutes, with fastest transformation observed in swimming pool water, likely due to the presence of chlorinating agents. In artificial skin experiments, sunscreen creams retained approximately 70-80% of *E*-EHMC, whereas hair oil formulations exhibited more extensive degradation. These findings raise concern about the diminished photoprotective efficacy of EHMC in sunscreen cream and its potential health risks, suggesting the need for regulatory reassessments.

A green, sensitive SPME-GC-MS method using acetylation derivatization was optimized for nine bisphenol analogues (BPA, BPAF, BPF, BPE, BPG, BPB, BPZ, BPM, and BPC2) across drinking water, environmental waters and waste waters, improving volatility and stability for trace analysis. The method achieved excellent linearity ($R^2 > 0.999$) and low detection limits in the low $\mu\text{g L}^{-1}$ range. Application of this method to 19 water samples revealed the presence of seven bisphenols, with concentration reaching up to 3.4 ng L^{-1} . BPE was detected at the highest concentration, and BPA was the frequently detected compound. This study provides a sensitive and environmentally sustainable approach for bisphenol analysis in complex water matrices.

The study of microplastics focused on the use of Py-GCMS to analyze MPs in an *in vitro* colon model. To ensure accurate detection of MPs amidst complex biological matrices, the Fenton oxidation method was employed for the degradation of organic matter, facilitating the extraction and quantification of PE and PS MPs. However, even after oxidation residual organic matter, specially lipids persisted. Nevertheless, PE and PS could be identified by typical pyrolyzates pattern and specific markers and quantified in the treated colon model samples in the two days of fermentation. Exposure to MPs induced significant shifts the gut microbiota with the overgrowth of opportunistic bacteria such as *Enterobacteriaceae* and *Desulfovibrio spp.* and a reduction in beneficial taxa like *Bifidobacterium* and *Faecalibacterium*. Together, these microbiomes and volatilome shifts provide mechanistic evidence that PE/PS MPs promote colon ecosystem changes consistent with dysbiosis within 24-48 hours under controlled human-microbiota fermentation. The health implication of these microbial changes could be profound, suggesting that MPs contribute to a range of diseases beyond the digestive system.

Despite the overall robustness of the developed/optimized methodologies, several methodological limitations should be acknowledged. First, matrix complexity represented a major analytical challenge, particularly for seawater rich in organic matter and for biological fermentation samples, where co-extracted lipids and proteins could not be completely removed and occasionally affected extraction efficiency and signal stability. Second, for UV filters and bisphenols, matrix induced signal suppression or enhancement remained unavoidable in some samples, despite optimized clean-up and calibration strategies. Third, in microplastics analysis, incomplete removal of residual organic material after Fenton oxidation limited absolute quantification accuracy, especially at low particle concentrations. In addition, the restricted availability of certified

reference materials and harmonized protocols for microplastic measurements constrained insight, cannot fully reproduce the complexity and variability of natural aquatic environments and human gastrointestinal systems.

Despite these limitations, the analytical strategies developed in this thesis offer a solid foundation for future methodologies advances and practical applications. The SPE-GC-MS/MS and SPME-GC-MS workflows may be readily implemented in environmental monitoring laboratories for large-scale surveillance of the UV-filters and bisphenols in coastal, freshwater and wastewater systems. These methods are well suited for regulatory compliance testing, long-term trend analysis, and human exposure assessment. The Py-GC-MS protocol may be further refined and standardized for routine evaluation of microplastics contamination in biological, food and environmental matrices.

Future research should focus on improving matrix cleanup efficiency through advanced sorbents, multidimensional separation techniques, and hybrid extraction approaches. The integration of high-resolution mass spectrometry may facilitate non-target screening of transformation products and unknown contaminants. In addition, the development of certified reference materials and standardized protocols for microplastics analysis remains a priority. Coupling chemical analysis with toxicological, microbiome, and epidemiological studies will further strengthen understanding of contaminants driven health effects and supportive evidence based risk assessment.

In conclusion, this thesis provides validated, sensitive, and environmentally sustainable analytical tools for investigating UV filters, bisphenols, and microplastics in complex environmental and biological systems. By critically addressing current methodological constraints and outlining realistic pathways for future development, validation, and application, this work strengthens the role of analytical chemistry in environmental protection, public health surveillance, and regulatory decision-making. The methodologies and findings presented here contribute to advancing contaminant monitoring and support informed strategies for mitigating the risks associated with emerging chemical pollutants.

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