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Review

An Overview of the Analytical Methods for the Determination of Organic Ultraviolet Filters in Cosmetic Products and Human Samples

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Abstract: UV filters are a group of compounds commonly used in different cosmetic products to absorb UV radiation. They are classified into a variety of chemical groups, such as benzophenones, salicylates, benzotriazoles, cinnamates, p-aminobenzoates, triazines, camphor derivatives, etc. Different tests have shown that some of these chemicals are absorbed through the skin and metabolised or bioaccumulated. These processes can cause negative health effects, including mutagenic and cancerogenic ones. Due to the absence of official monitoring protocols, there is an increased number of analytical methods that enable the determination of those compounds in cosmetic samples to ensure user safety, as well as in biological fluids and tissues samples, to obtain more information regarding their behaviour in the human body. This review aimed to show and discuss the published studies concerning analytical methods for the determination of organic UV filters in cosmetic and biological samples. It focused on sample preparation, analytical techniques, and analytical performance (limit of detection, accuracy, and repeatability).

Keywords: analytical methodologies; cosmetics products; human samples; organic ultraviolet filters; sample preparation

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1. Introduction

In recent decades, there has been a progressive increase in UV radiation due to the depletion of the stratospheric ozone layer. This promotes an increase in the number of harmful effects on human health such as skin burns, skin photoaging, damage to the skin's immunological system, pterygium, or skin cancer [1,2]. Accordingly, the number of personal care products containing UV filters has increased rapidly to protect human skin from damaging exposure to sunlight. The currently estimated volume production of UV filters reaches 26.9 million tons [3]. UV filters are frequently added to all types of personal care products such as lotions, shampoos, creams, aftershave products, make-up products, etc. [4–6].

The European Union (EU) Regulation 1223/2009—Cosmetics Regulation 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" [7]. UV filters are classified into two groups: organic (chemical) UV filters, which absorb UV light, as well as inorganic (physical) UV filters, which reflect and scatter UV radiation. Chemical UV filters are organic molecules capable of absorbing high UV-A and UV-B range radiation. The UV filters have one or more benzene rings and sometimes are conjugated with carbonyl groups [8]. They can be classified into different groups according to their chemical structure: benzophenone derivatives, p-aminobenzoic acid and its derivatives, salicylates, cinnamates, camphor derivatives, triazine derivatives, benzotriazole derivatives, benzimidazole derivatives, and others (Table 1) [9]. One of the most widely used family

of UV filters are benzophenones, in particular BP-3, which in 2012 was classified by the US Environmental Protection Agency (US EPA) as "high production volume chemical" [3]. The scale of the problem of the existence of UV filters in the environment was presented by Astle et al. [3], who performed research among Swiss sunbathers on the use of UV filters during one tourist season. On their basis, it was estimated that about 1249 kg of ethylhexyl methoxycinnamate, 152 kg of octocrylene, 145 kg of 4-MBC, and 122 kg of avobenzene were released into Lake Zürich. Therefore, these compounds are the most frequently determined UV filters.

To protect consumers' health, the substances that can be used as UV filters in personal care products and their maximum allowed concentrations are strictly defined in each country [8]. The European Union regulations permit the use of 29 UV filters in cosmetics in concentrations ranging from 2 to 25% (Table 1). However, only two are inorganic (titanium dioxide and zinc oxide) [7]. Organic UV filters have a hydrophilic or lipophilic character and most of them are classified as water-resistant [8].

Despite the limitations on their use in UV filters, there are no established official analytical methods for the determination of these compounds in cosmetics products. However, to maintain the safety and adequate effectiveness of products containing UV filters, analytical methods should be developed to control the content of UV filters in them [10].

Moreover, due to the daily use of cosmetics containing UV filters, such compounds are absorbed through the skin into the body, where they can be metabolized and eventually bioaccumulated and/or excreted. The dermal absorption may result in harmful health effects like dermatitis but also more serious effects, such as mutagenic, cancerogenic, and/or estrogenic activity [11]. Therefore, because of the adverse effects of UV filters on human health and their potential bioaccumulation, such biological samples as urine, plasma, breast milk, semen, or tissues must be checked for their presence.

In this context, this review aimed to provide a comprehensive overview of the developments related to the determination of UV filters in cosmetic samples and biological fluids and tissues, with special emphasis on sample preparation and analytical techniques, as well as the achieved detection limits, accuracy, and repeatability.

2. Analytical Methods for UV Filter Determination in Cosmetic Samples

2.1. Sample Preparation

Cosmetic sample preparation depends on sample type, target analytes, and the technique that is to be used. In general, the preparation of a cosmetic sample does not require a complex pre-treatment sample. This is because the UV filter content in the cosmetic samples is at a sufficiently high level for the sample treatment not to require the extraction and concentration steps. Additionally, in most cases (approximately 90%), liquid chromatography is used for analysis, which enables direct analysis of matrices such as cosmetics. It was alleged that in recent decades the methods of determining UV filters in cosmetics have not been modified too much [11,12].

The initial preparation of the sample consists of dissolving a cosmetic sample in a carefully selected solvent (typically ethanol, methanol, ethyl acetate, water, tetrahydrofuran). The step of dissolving the cosmetic sample may be preceded by homogenisation. Depending on the cosmetic product's type (i.e., consistency), the next steps in the procedure may include sonicating the sample for a few minutes (5–30 min, 40 °C) [10,13–33], magnetic mixing [34,35], mechanical shaking [20,36], vortexing (3–4 min), [25,29,32,37], or centrifuging (1–20 min, 3500–14,800 rpm) [14,19,20,25,27,29,32,33], which can help accelerate the solubilisation. The obtained supernatant is often filtered as well (e.g., 0.45 µm nylon membrane filter) [10,13–18,21–26,37] and/or evaporated [19,25,27,29,33,38].

Table 1. List of compounds that can be allowed as organic UV filters in cosmetic products according to the European Union legislation.

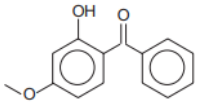
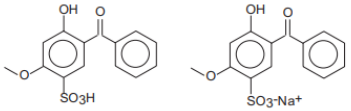
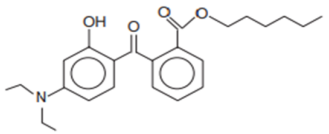
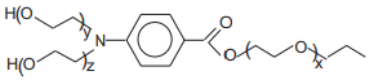
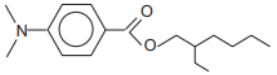
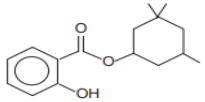
Chemical Name	INCI Name [a]	Abbreviation	CAS Number	Structure	Max. Concentration (%)	Log $K_{o/w}$ [a]	p_{Ka} [a]	Solubility (g/L) [a,b]
Benzophenone derivatives								
2-Hydroxy-4-methoxybenophenone/Oxybenzone	Benzophenone-3	BP-3	131-57-7		10	3.79	7.56	0.21
2-Hydroxy-4-benzophenone-5-sulfonic acid and its sodium salt/Sulisobenzoate	Benzophenone-4, Benzophenone-5	BP-4, BP-5	4065-45-6/6628-37-1		5 (as acid)	0.37	−0.70	0.65
Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-hexylester	Diethylamino Hydroxybenzoyl Hexyl Benzoate	DHHB	302776-68-7		10	6.54	7.29	$9.5 \cdot 10^{-4}$
p-Aminobenzoic acid derivatives								
Ethoxylated ethyl-4-aminobenzoate	PEG-25 PABA	PEG-25 PABA	116242-27-4		10	−0.66	-	-
2-Ethylhexyl-4-(dimethylamino)benzoate/Padimate O (USAN:BAN)	Ethylhexyl Dimethyl PABA	OD-PABA	21245-02-3		8	6.15	2.39	0.0021
Salicylates								
Benzoic acid, 2-hydroxy-3,3,5-trimethylcyclohexyl ester/Homosalate	Homosalate	HS	118-56-9		10	6.16	8.09	0.02

Table 1. *Cont.*

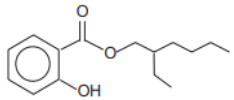
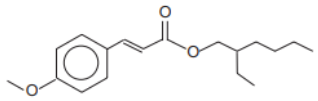
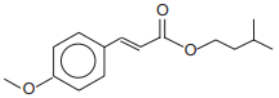
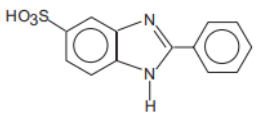
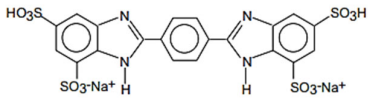
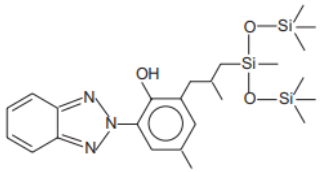
Chemical Name	INCI Name [a]	Abbreviation	CAS Number	Structure	Max. Concentration (%)	Log $K_{o/w}$ [a]	p_{Ka} [a]	Solubility (g/L) [a,b]
2-Ethylhexyl salicylate/Octisalate	Ethylhexyl Salicylate	EHS	118-60-5		5	5.97	8.13	0.028
Cinnamates								
2-Ethylhexyl-4-methoxycinnamate/Octinoxate	Ethylhexyl Methoxycinnamate	OMC	5466-77-3		10	5.8	-	0.15
Isopentyl-4-methoxycinnamate/Amiloxate	Isoamyl p-Methoxycinnamate	IMC	71617-10-2		10	4.33	-	0.06
Benzimidazole derivatives								
2-Phenylbenzimidazole-5-sulfonic acid and its potassium, sodium, and triethanolamine salts/Ensulizole	Phenylbenzimidazole Sulfonic Acid	PMDSA	27503-81-7		8 (as acid)	−0.16	−0.87	0.26
Sodium salt of 2,2′-bis(1,4-phenylene)-1H-benzimidazole-4,6-disulfonic acid)/Bisdisulizole disodium (USAN)	Disodium Phenyl Dibenzimidazole Tetrasulfonate	DPDT	180898-37-7		10 (as acid)	−6.79	−0.27	0.5
Benzotriazole derivatives								
Phenol,2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl)	Drometrizole Trisiloxane	DTS	155633-54-8		15	10.38	1.2	$5.5 \cdot 10^{-10}$

Table 1. *Cont.*

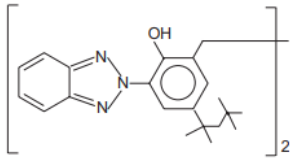
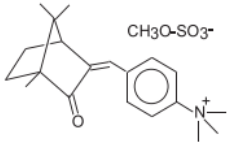
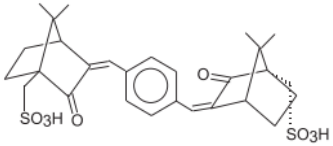
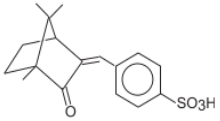
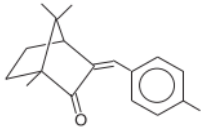
Chemical Name	INCI Name [a]	Abbreviation	CAS Number	Structure	Max. Concentration (%)	Log $K_{o/w}$ [a]	p_{Ka} [a]	Solubility (g/L) [a,b]
2,2′-Methylene-bis(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethyl-butyl)phenol)/Bisoctrizole	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol	MBP	103597-45-1		10	12.46	7.56	$3 \cdot 10^{-8}$
Camphor derivatives								
N,N,N-Trimethyl-4-(2-oxoborn-3-ylidenemethyl)anilinium methyl sulfate	Camphor Benzalkonium Methosulfate	CBM	52793-97-2		6	0.28	-	0.007
3,3′-(1,4-Phenylenedimethylene) bis(7,7-dimethyl-2-oxobicyclo-[2,2,1]hept-1-yl-methanesu fonic acid) and its salts/Ecamsule	Terephthalylidene Dicamphor Sulfonic Acid	PDSA	92761-26-7, 90457-82-2		10 (as acid)	3.83	−1.05	0.014
Alpha-(2-Oxoborn-3-ylidene)-toluene-4-sulphonic acid and its salts	Benzylidene Camphor Sulfonic Acid	BCSA	56039-58-8		6 (as acid)	2.22	−0.7	0.038
3-(4-Methylbenzylidene)-d1 camphor/Enzacamene	4-Methylbenzylidene Camphor	4-MBC	38102-62-4/ 36861-47-9		4	4.95	-	0.0051

Table 1. *Cont.*

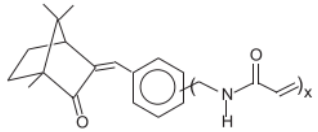
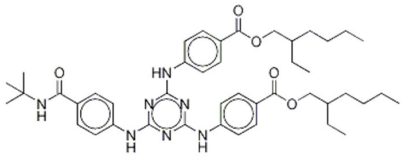
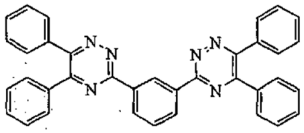
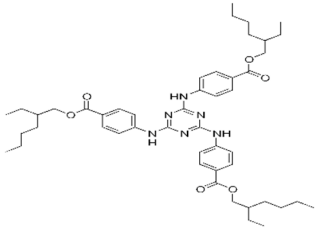
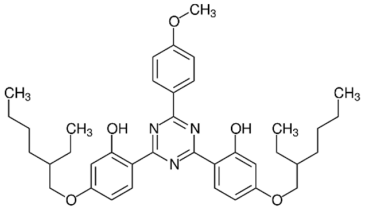
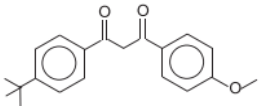
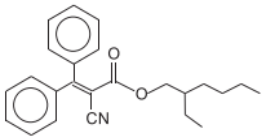
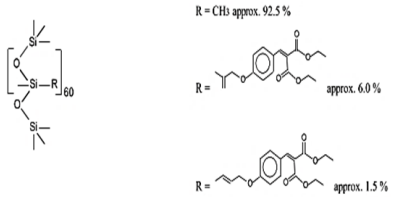
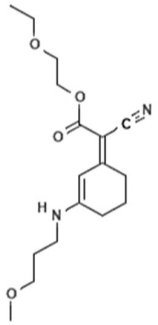
Chemical Name	INCI Name [a]	Abbreviation	CAS Number	Structure	Max. Concentration (%)	Log $K_{o/w}$ [a]	p_{Ka} [a]	Solubility (g/L) [a,b]
Polymer of N-{(2 and 4)-[(2-oxoborn-3-ylidene)methyl-]benzyl} acrylamide	Polyacrylamidomethyl Benzylidene Camphor	PBC	113783-61-2		6	-	-	-
Triazine derivatives								
Benzoic acid, 4,4-((6-((4-(((1,1-dimethylethyl)amino)carbonyl) phenyl)amino)-1,3,5-triazine-2,4-diyl)diimino)bis-, bis (2-ethylhexyl) ester/ Iscotrizinol (USAN)	Diethylhexyl Butamido Triazone	DBT	154702-15-5		10	14.03	3.04	$4.6 \cdot 10^{-7}$
3,3′-(1,4-Phenylene)bis(5,6-diphenyl-1,2,4-triazine)	Phenylene Bis-Diphenyl triazine	-	55514-22-2		5	-	-	-
2,4,6-Trianilino-(p-carbo-2′-ethylhexyl-1′-oxy)-1,3,5-triazine	Ethylhexyl Triazone	ET	88122-99-0		5	17.05	3.17	-
2,2′-(6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-diyl)bis(5-((2-ethylhexyl)oxy)phenol)/ Bemotrizinol	Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	EMT	187393-00-6		10	8.03	6.37	$4.9 \cdot 10^{-8}$

Table 1. *Cont.*

Chemical Name	INCI Name [a]	Abbreviation	CAS Number	Structure	Max. Concentration (%)	Log $K_{o/w}$ [a]	p_{Ka} [a]	Solubility (g/L) [a,b]
				Others				
1-(4-tert-Butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione/Avobenzene	Butyl Methoxydibenzoyl-methane	BMDBM	70356-09-1		5	4.51	9.74	0.037
2-Cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester/Octocrilene	Octocrylene	OC	6197-30-4		10 (as acid)	6.88	-	$2 \cdot 10^{-4}$
Dimethicodiethylbenzalmalonate	Polysilicone-15	BMP	207574-74-1		10	-	-	-
2-ethoxyethyl(2Z)-2-cyano-2-[3-(3-methoxy-propylamino) cyclohex-2-en-1-ylidene]acetate	Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate	-	1419401-88-9		3	-	-	-

[a] From Cadena-Aizaga M.I. et al. [39]. [b] Solubility in water at 25 °C.

These procedures are aimed at completely dissolving the sample or leaching the target analytes (e.g., in case of difficult-to-dissolve samples such as wax-balms, lipsticks, or foundations containing insoluble compounds). The achieved high recoveries (Table 2), amounting from 80 to 113%, confirm the effectiveness of these procedures.

Table 2. Published studies on UV filters determination in cosmetic samples.

UV Filters	Matrix	Analytical Technique	Analytical Performance [a]	Ref.
BP-3, IMC, MBC, DHHB, OC, EDP, BDM, EMC, EHS, HS, DBT, ET, DTS, MBP, EMT	Sunscreens, facial creams, lip balms, aftershave creams	LC-UV/Vis; type of column: C_{18}; column temperature: 60 °C; mobile phase: ethanol/formic acid (aq) mobile phase modifier: hydroxypropyl-β-cyclodextrin (HP-β-CD)	LOD: 0.02–0.22 μg mL^{-1} LOQ: 0.07–0.74 μg mL^{-1} R: 98–104% RSD: 0.9–7.1%	[10]
PMDSA, BP-4, BP-3, MBC, DHHB, EMC, OC, MBP, EMT, ET, BDM	Emulsion, oil	HPLC-UV/Vis; type of column: C_8 or C_{18} or C_{16}; column temp.: 35 °C; mobile phase: gradient acetonitrile/perchloric acid (aq) or isocratic methanol/acetonitrile or isocratic methanol/perchloric acid	LOD: 0.1–1.2 μg mL^{-1} LOQ: no data R: 93.9–103.4% RSD: 0.2–1%	[13]
BP-1, BP-2, BP-3	Emulsion	MEKC-UV/Vis; type of capillary: a 51 cm uncoated fused-silica; surfactant: sodium tetraborate containing sodium dodecyl sulfate	LOD10^{-8}–3.90 $\cdot 10^{-7}$ mol/L LOQ: no data R: 89.5–102.5% RSD: 1.14–8.09%	[14]
PMDSA, PABA, BP-4, BP-3, IMC, MBC, OC, EMC, HS, EHS, MBBT	Creams, lotions, foundation, loose powder, lipstick	HPLC-UV/Vis; type of column: C_{18}; column temp.: 30 °C; mobile phase: gradient methanol/tetrahydrofuran/perchloric acid (aq)	LOD: 200–500 ng mL^{-1} LOQ: 700–6700 ng mL^{-1} R: 98.5–102.2% RSD: 0.51–1.72%	[15]
PMDSA, BP-3, IMC, DHHB, OC, EMC, EHS, BDM, DBT, ET, MBP, EMT	Emulsion, sticks, powder	HPLC-UV/Vis; type of column: C_{18}; column temp.: 40 °C; mobile phase: gradient ethanol/ 1% phosphoric acid (aq)	LOD: 0.04–1.66 μg mL^{-1} LOQ: 0.13–5.52 μg mL^{-1} R: 97–101.4% RSD: 0.38–2.42%	[16]
HS, EDP, EHC, EHS, MBC, BDM, BP-3, OC, PHBA, BC	Cream, milk, lotion, oil, lipstick	DART-MS (ESI^+)	LOD: 2.5–460 μg g^{-1} LOQ: no data R: 71–120% RSD: 4–30%	[17]
EMC, IMC, EHS, MBC, BP-3, EDP, OC, BDM	Cream, lotion, spray	HPLC-UV/Vis; type of column: C_{18}; column temp.: 30 °C; mobile phase: gradient acetonitrile/acetic acid (aq)	LOD: 0.03–1.5 mg L^{-1} LOQ: 0.08–4.6 mg L^{-1} R: 98–102% RSD: 0.97–6.1%	[18]
BP-4, BP-3, ODP, OMC, EHS	Cream, lotion, lipstick, foundation	HPLC-UV/Vis; type of column: C_{18}; column temp.: 40 °C; mobile phase: gradient methanol/pure water (80:20; *v*/*v*)	LOD: 1–100 ng L^{-1} LOQ: 4–340 ng L^{-1} R: 98–102% RSD: 4–5.2%	[19]
OC	Emulsion	SWV/mercury electrode; a mixture of Britton–Robinson (BR) buffer and ethanol (7:3; *v*/*v*) as the supporting electrolyte	LOD: no data LOQ: no data R: 9.7–106% RSD: 1–3.42%	[20]
EMC, BP-3, EHS, OC	Emulsion	LC-UV/Vis; type of column: C_{18}; mobile phase: methanol/water (85:15; *v*/*v*)	LOD: no data LOQ: no data R: 99.67–101% RSD: 0.044–1.5%	[21]

Table 2. *Cont.*

UV Filters	Matrix	Analytical Technique	Analytical Performance [a]	Ref.
BDM, BP-3, EMC	Cream	HPTLC-DS.; type of column: $C_{18\ or}$ silica gel; mobile phase: acetonitrile/water (18:2) or cyclohexane/diethyl ether/n-hexane/acetone (14:2:1:2)	LOD: no data LOQ: no data R: 92.7–102.4% RSD: no data	[22]
PABA, PMDSA, BP-3, MBC, BP-4, OC, EDP, EMC, BDM, HS, EHS, DBT, ET, DTS	Cream	HPLC-UV/Vis; type of column: C_{18}; mobile phase: gradient ethanol/phosphate buffer	LOD: 0.01–1.99 mg L^{-1} LOQ: 0.02–6.02 mg L^{-1} R: 90.91–109.98% RSD: 0.16–12.69%	[23]
BP-3, BP-4	Shampoo, gel, perfume, cream	MEKC-UV/Vis; type of capillary: a 64.5 cm uncoated fused-silica; surfactant: sodium dodecyl sulphate	LOD: 0.91–2.26 µg mL^{-1} LOQ: 2.72–6.79 µg mL^{-1} R: 90.4–107.4% RSD: 5.7–12%	[24]
BP-1, BP-2, BP-3, BP-4, BP-6, BP-8, OC, EMC, PABA	Lotion, cream	MEKC-UV/Vis; type of capillary: a 30.2 cm uncoated fused-silica; surfactant: sodium dodecyl sulfate/γ-cyclodextrin	LOD: no data LOQ: no data R: 95.08–104.57% RSD: no data	[25]
PABA, BP-3, IMC, MBC, OC, EDP, EMC, BDM, EHS, HS	Cream	HPLC-UV/Vis; type of column: C_{18}; column temp.: 35 °C; mobile phase: isocratic ethanol/acetic acid (aq) (70:30; *v*/*v*)	LOD: 0.1–2 µg mL^{-1} LOQ: 0.5–5µg mL^{-1} R: no data RSD: no data	[26]
BP, BP-3, BP-1, HBP	Cream	MEKC-UV/Vis; type of capillary: a 60 cm uncoated fused-silica; surfactant: sodium dodecyl sulfate	LOD: 3.9–6.7 ng mL^{-1} LOQ: 13–22.3 ng mL^{-1} R: 80.2–117.7% RSD: no data	[27]
BP-3, EMC, OC, EHS, MBC, EDP	Cream, lipstick, blemish balm cream	LTP-MS	LOD: no data LOQ: no data R: no data RSD: 0.8–28.6%	[28]
PMDSA, BP-2, BP-1, BP-8, BP, BP-6, BP-3, EHS, BP-10, HS, IMC, MBC, DHHB, BDM, BP-12	Lotion, cream, lipstick	HPLC-MS/MS (ESI); type of column: C_{18}; column temp.: 30 °C; mobile phase: gradient methanol/0.1% ammonium hydroxide (aq)	LOD: 2–20 mg kg^{-1} LOQ: 5–50 mg kg^{-1} R: 86.9–103.5% RSD: 1–6.8%	[29]
EHS, EMC, BP-3, OC, EMT, BDM, DHHB, ET, DBT	Cream	UHPSFC-PDA; type of column: Torus 2-PIC; column temp.: 40 °C; mobile phase: gradient CO_2/methanol/water/ammonium acetate	LOD: 0.2–1.7 mg kg^{-1} LOQ: 1–10.8 mg kg^{-1} R: 97.5–103.2% RSD: 0.7–1.6%	[30]
BP-1, BP-2, BP-3, BP-8, HBP	Toothpaste, shampoo, face cleansers, sunscreens, body lotions, gels, hair gels,lotions, mask, hand sanitizer	HPLC-MS/MS (ESI^-); type of column: C_{18}; column temp.: 40 °C; mobile phase: gradient methanol/acetonitrile/water	LOD: 0.002–0.197 ng mL^{-1} LOQ: 0.001–0.059 ng mL^{-1} R: 61.9–116% RSD: no data	[31]
BP-1	Nail product	GC-MS/MS (EI^+); type of column: ZB-SemiVolatiles; oven temp.: 40 °C/2 min—5 °C/1 min to 65 °C—50 °C/1 min to 300 °C/5 min	LOD: 18.3–2370 µg g^{-1} LOQ: no data R: 101–105% RSD: 0.69–1.13%	[32]
BDM, EMT, OMC, OC, ET	Lotion	HPLC-UV/Vis; type of column: C_{18}; mobile phase: acetonitrile/0.25% formic acid (aq)	LOD: 15 ng mL^{-1} LOQ: no data R: 88.1–104.7% RSD: 0.8–5.4%	[33]
BDM	Emulsion	LC-UV/Vis; type of column: C_{18}; column temp.: 42 °C; mobile phase: acetonitrile/0.5% phosphoric acid (aq)	LOD: 0.05796 µg mL^{-1} LOQ: 0.19322 µg mL^{-1} R: no data RSD: 0.46–2.83%	[34]

Table 2. *Cont.*

UV Filters	Matrix	Analytical Technique	Analytical Performance [a]	Ref.
EMC, MBC, BP-1, BP-2, BP-6, BP-4, OC, PABA, EDP, EHS, HS, IMC, BP-3, BP-8, BS, MA	Cream, nail polish, lipstick, hair gel	GC-MS/MS (EI^+); type of column: SLB-5 ms; oven temp.: 100 °C/1 min—25 °C/1 min—290 °C/5 min	LOD: 0.0027–0.56 μg g^{-1} LOQ: 0.009–1.9 μg g^{-1} R: 37.4–110.5% RSD: 3.9–9.1%	[35]
ET	Cream, lotion	TLC-DS.; type of layer: silica gel; mobile phase: cyclohexanediethyl ether (1:1)	LOD: 0.03 μg $spot^{-1}$ LOQ: 0.1 μg $spot^{-1}$ R: 95–105% RSD: 4.5–5%	[36]
PMDSA, BDM, OC, EHS	Cream	HTLC; type of column: C_{18}; column temp.: 150–200 °C; mobile phase: isocratic methanol/water	LOD: no data LOQ: no data R: 90.3–113.2% RSD: 2.8–5%	[37]
EMC, MBC, BP-1, BP-2, BP-6, BDM, BP-4, PMDSA, MA, OC, EDP, IMC, BP-3, BP-8,	Lipsticks, hair gel, cream, nail polish	HPLC-MS/MS; type of column: C_{18}; oven temp.: 30 °C; mobile phase: gradient methanol/0.1% formic acid/ammonia (aq)	LOD: 0.00039–0.031 μg g^{-1} LOQ: 0.0013–0.1 μg g^{-1} R: 81.7–102% RSD: 4.5–13%	[38]
BDM, BP-3, EMC, EMT	Emulsion	HPLC-UV/Vis; type of column: C_{18}; column temp.: 25 °C; mobile phase: gradient tetrahydfofuran/acetonitrile/acetic acid (aq)	LOD: no data LOQ: no data R: 99.2–104.8% RSD: no data	[40]
BP-4	Shampoo	TLC-UV/Vis; type of layer: silica gel 60 plates; mobile phase: acetate/ethanol/water/phosphate buffer (15:7:5:1; *v*/*v*/*v*/*v*)	LOD: 0.03 μg $spot^{-1}$ LOQ: 0.1 μg $spot^{-1}$ R: 100–103% RSD: 0.58–1.99%	[41]
EHS, EMC, BP-3, OC, BDM, DHHB, ET, DBT	Cream	SFC-UV/Vis; type of column: 2-ethyl pyridine; column temp.: 30 °C; mobile phase: gradient CO_2/methanol/ethanol (97:1.5:1.5)	LOD: no data LOQ: no data R: no data RSD: 0.6–2%	[42]

[a] LOD and LOQ expressed as: *w*/*w* when referred to sample or *w*/*v* when referred to sample solution.

Despite the UV filters being the basic components of the samples, no special extraction techniques are needed. However, some authors proposed the use of extraction techniques such as pressurised liquid extraction [35,38], cloud point extraction [14], dispersive liquid–liquid microextraction [27], or hollow fibre liquid-phase microextraction [19].

2.2. Analytical Techniques

Since the UV filters are part of the cosmetic products, their determination by direct measurement without a prior separation step is impossible. As such, chromatography methods are typically used. The most common chromatographic technique for determining UV filters is liquid chromatography; this is because UV filters have very high boiling points. In the majority of publications, the reversed-phase liquid chromatography coupled with a UV/Vis spectrometry detector with a single wavelength or with a diode-array is commonly used for this purpose. The application of a diode-array detector makes it possible to receive the whole UV spectrum for all peaks. The most used stationary phase is the traditional octadecylsilica type (C18), but octysilica (C8) and amide (C16) have been used as well [9]. In the case of reversed-phase separations, the most used solvents include water, methanol, tetrahydrofuran, acetonitrile, or their mixtures. The more environmentally friendly analytical methods include using the ethanol–water mixture in the mobile phase [6,12,19,22]. Isocratic or gradient elution modes are practised as well. Some substances can be added to the eluent to cut back peak tailing, such as acetic acid in the case of BP-3 [14,35]. Such reagents as phosphate, sodium acetate, and ammonium

acetate are used for buffering. Hydroxypropyl-β-cyclodextrin is used as a mobile phase modifier to improve the resolution between varied analytes [6].

Therefore, gas chromatography is used in derivatization procedures with silylating reagents that can increase UV filter volatility, as well as sensitivity. Some publications [32,35] describe the use of gas chromatography coupled with mass spectrometry with electron impact, with N,O-Bis(trimethylsilyl) trifluoroacetamide and acetic anhydrite used as the derivatizing reagents.

Apart from liquid and gas chromatography, there are also a few other separation techniques. One of them is micellar electrokinetic chromatography [14,24,25,27], which utilises uncoated silica capillaries and sodium dodecyl sulphate as a surfactant. Others include thin-layer chromatography [22,36,37,41], supercritical fluid chromatography [30,42], and square wave voltammetry [20]. Table 2 shows the published reports on the determination of individual UV filters, including the sample preparation step and the analytical methodology, as well as the results obtained in terms of the limits of quantification, recovery method, and its precision.

3. Analytical Methods for UV Filter Determination in Biological Samples

Upon classifying published studies dealing with the determination of UV filters in human samples according to the studied matrix (Tables 3–5), it is clearly visible that the most studied biological matrix is urine (~61%), followed by blood, plasma, or serum (~20%). Other matrices such as milk (~7%), tissues (~5%), and nail, semen, or saliva (~8%) have only been analysed intermittently (Figure 1).

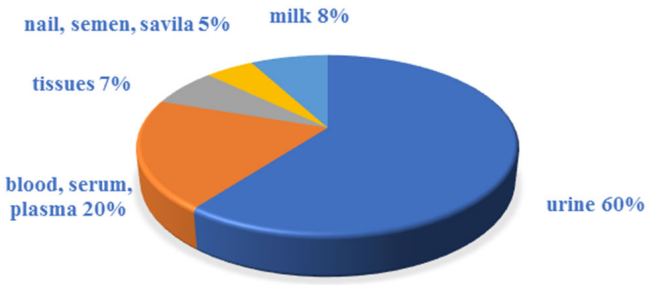


Figure 1. Biological sample types in the determination of UV filters.

To date, most research work is focused on the analysis of BP-3 and its metabolites, which have been widely determined in all types of biological samples. Other UV filters that have been analysed, albeit less often, include EMC, OMC, PABA, BDM, EDP, ES, HS, TDS, etc.

3.1. Sample Preparation

To determine UV filters in biological samples, the extraction (~75%) and microextraction (~25%) techniques have been used (Figure 2). Extraction techniques include liquid–liquid extraction (LLE) (~28%), solid-phase extraction (SPE) (~28%), fabric phase sorptive extraction (FPSE) (~5%), as well as the less frequently used accelerated solvent extraction (ASE); microwave-assisted digestion/extraction (MAE); microporous membrane liquid-liquid extraction (MMLLE); matrix solid-phase dispersion (MSPD); sequential injection solid-phase extraction (SI SPE); Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction (QuEChERSExtraction); solid–liquid extraction (SLE); ultrasound-assisted extraction (UAE); and ultrasound-assisted dispersive solid-phase extraction (USAD-SPE) (each ~2%).

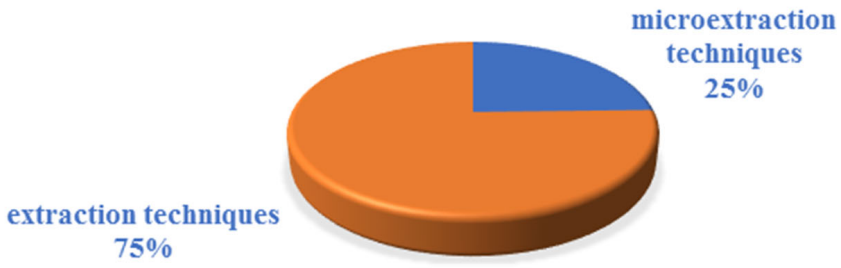


Figure 2. Division of analytical techniques into extraction and microextraction techniques.

In the last decades, a gradual increase in the use of microextraction methods for the isolation and enrichment of analytes in the tested samples has been observed. In the work of Jiménez-Díaz et al. from 2014 [43] on methods for determining UV filters in human samples, the contribution of microextraction methods was only about 7%. Microextraction techniques include the dispersive liquid–liquid microextraction (DLLME) (~10%), as well as the less frequently employed air-assisted liquid–liquid microextraction (AALLME), bar adsorptive microextraction (BAμE), hollow-fibre liquid-phase microextraction (HFLPME), microextraction by packed sorbent (MEPS), stir bar sorptive extraction (SBSE), single-drop microextraction (SDME), solid-phase microextraction (SPME), microextraction using a monolithic stirring extraction unit (MUMSEU), and vortex-assisted dispersive liquid–liquid microextraction (VADLLME) (each of them accounts for ~2%) (Figure 3).

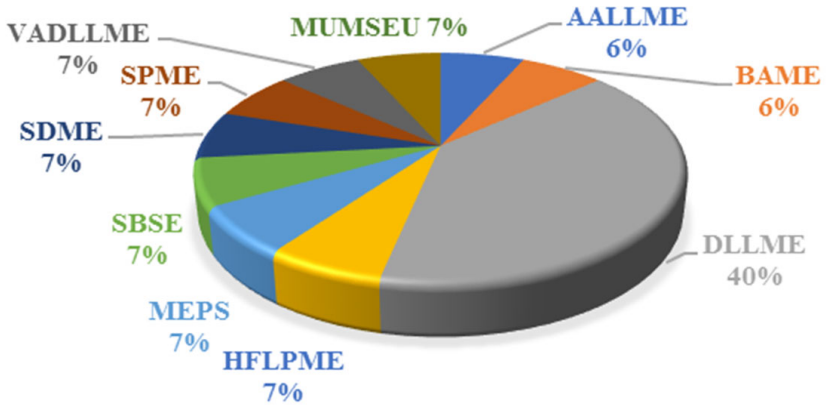


Figure 3. Microextraction techniques used for the determination of UV filters in biological samples.

Urine is the most frequently analysed sample. In urine, the compounds usually occur in free and conjugated forms; hydrolysis is often required to determine their total content (free plus conjugated). Without the hydrolysis step, it is only possible to determine the content of the free ones. The difference between free and conjugated content gives the total conjugated content. Older studies typically used 6 M hydrochloric acid to hydrolyse the bounded compounds [44,45]. Today, enzymatic hydrolysis is achieved by incubating a urine sample with β-glucuronidase or with β- glucuronidase/sulfatase (under specific conditions such as pH, temperature, and time) [46–68]. After enzymatic hydrolysis, the enzyme is denatured by treated with cold acetonitrile, methanol, or acetic acid to stop the reaction and then separated by centrifugation. The supernatant undergoes the next sample preparation step.

Table 3 summarises the extraction techniques used in the methods for determining UV filters in urine published in the literature. Liquid–liquid extraction (LLE) [51,55,57,58,63,66,69] and solid-phase extraction (SPE) [46–50,56,59,62,64,65,70–72] are the most popular extraction techniques used to determine the UV filters. Accelerated solvent extraction (ASE) [62],

fabric phase sorptive extraction (FPSE) [73], microporous membrane liquid–liquid extraction (MMLLE) [74], and sequential injection solid-phase extraction (SI SPE) [75] have been employed as well. However, microextraction techniques are also used to reduce solvent consumption and increase concentration factors. Microextraction techniques include air-assisted liquid–liquid microextraction (AALLME) [68], bar adsorptive microextraction (BAμE) [76], dispersive liquid–liquid microextraction (DLLME) [61,77], hollow-fibre liquid-phase microextraction (HFLPME) [55], microextraction by packed sorbent (MEPS) [78], stir bar sorptive extraction (SBSE) [53], single-drop microextraction (SDME) [52], solid-phase microextraction (SPME) [79], and vortex-assisted dispersive liquid-liquid microextraction (VADLLME) [67].

Table 3. Published papers on UV filters determination in urine.

UV Filters	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
BP-3	SPE (C_8)	HPLC-UV/Vis; type of column: C_{18}; mobile phase: isocratic methanol/water (70:30)	No data	Total content	[44]
BP-3	SPE (Bond Elut Certify LRC)	UPLC-MS/MS (ESI^-); type of column: Kinetex Phenyl-Hexyl; column temp.: 35 °C; mobile phase: water/acetonitrile/acetic acid (aq)	LOD: 0.3 ng mL^{-1} LOQ: 0.61–200 ng mL^{-1} R: 75.8–80.3% RSD: 0.3–8%	Total and free forms content	[46]
BP-3	Online SPE (RP_{18})	HPLC-MS/MS ($APCI^-$); type of column: RP_{18}; mobile phase: gradient methanol/water	LOD: 0.3–0.5 ng mL^{-1} LOQ: no data R: 97–105% RSD: 1.7–20%	Total and forms content	[47–49]
BP-3	SPE (C_{18})	HPLC-MS (APCI); type of column: C18-PFP; mobile phase: methanol/water	LOD: 0.2 ng mL^{-1} LOQ: no data R: 96% RSD: 9.03–11.7%	Total content	[50]
BP-1, BP-2, BP-8, 4-OH-BP	LLE (solvent: ethyl acetate)	HPLC-MS/MS (ESI^+ / ESI^-); type of column: C_{18}; mobile phase: methanol/water (90:10; *v*/*v*)	LOD: no data LOQ: 0.7–2.0 ng mL^{-1} R: 84–112% RSD: no data	Total content	[51]
BP-3	SDME (acceptor phase:[C6MIM][PF6]; 25 min; 900 rpm)	LC-UV; type of column: RP_{18}; mobile phase: ethanol/1% acetic acid aq (60:40; *v*/*v*)	LOD: 1.3 ng mL^{-1} LOQ: no data R: no data RSD: 6%	Free forms	[52]
BP, BP-OH, 2-OH-BP, BP-3, BP-10	SBSE (PDMS; 60 min; 500 rpm)	GC-MS; type of column: DB-5 ms; oven temp.: 40 °C/1 min—5 °C/ 1 min to 190 °C—15 °C/ 1 min to 280 °C/3 min	LOD: 0.05–0.1 ng mL^{-1} LOQ: 0.2–0.5 ng mL^{-1} R: 98.7–101.7% RSD: 1.5–4.8%	Free forms	[53]
BP, BP-OH, 2-OH-BP, BP-3, BP-10	HFLPME (toluene; 15 min; 500 rpm)	GC-MS (EI); type of column: DB-5 ms; oven temp.: 40 °C/1 min—5 °C/ 1 min to 190 °C—15 °C/1 min to 280 °C/ 4 min	LOD: 5–10 pg mL^{-1} LOQ: 20–50 pg mL^{-1} R: 89.3–100.2% RSD: 2.5–9.3%	Total content	[54]
BP-1, BP-3, BP-8, BP-2, 4-OH-BP	LLE (solvent; 50% MTBE/ethyl acetate)	HPLC-MS/MS (ESI^-); type of column: C_{18}; mobile phase: gradient methanol/water	LOD: 0.08–0.28 mg mL^{-1} LOQ: 0.28–0.9 mg mL^{-1} R: 85.2–99.6% RSD: 2.8–4.5%	Total content	[55]
BP-1, BP-3, BP-8, THB	SPE (C_{18})	LC-MS/MS (ESI^+); type of column: Mediterranean SEA 18; mobile phase: gradient methanol/water/0.1% formic acid aq	LOD: 1 ng mL^{-1} LOQ: 2–4 ng mL^{-1} R: 84–111% RSD: no data	Total content	[56]

Table 3. *Cont.*

UV Filters	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	LLE (solvent; 50% MTBE/ethyl acetate)	HPLC-MS/MS (ESI); type of column: C_{18}; mobile phase: gradient methanol/water	LOD: 0.013–0.28 ng mL^{-1} LOQ: no data R: 85.2–99.6% RSD: 1.4–4.5%	Total content	[57]
BP-1, BP-2, BP-3, BP-7, 4-OH-BP, 4-MBP, 4-MBC, 3-BC	LLE	On-line TurboFlow-LC–MS/MS; type of column: TurboFlow Cyclone P and Hypersil Gold aQ	LOD: 0.2–1.0 ng mL^{-1} LOQ: no data R: 77.1–108% RSD: 5.7–15.1%	Total and free form content	[58]
EDP	Automated SPE (C_{18} HD)	LC-MS/MS (ESI^+); type of column: Mediterranean SEA C_{18}; mobile phase: gradient methanol/acetonitryle/water/0.2% formic acid	LOD: 0.3–1.1 ng mL^{-1} LOQ: 0.9–3.5 ng mL^{-1} R: 91–107% RSD: no data	Total and free forms content	[59]
BP-3, OMC, OS, HS	LLE (solvent: acetonitrile)	HPLC-DAD; type of column: C_{18}; mobile phase: gradient methanol/water (75:25; *v*/*v*)	LOD: 0.03–0.2 µg mL^{-1} LOQ: 0.1–0.4 µg mL^{-1} R: 86.8–92.2% RSD: 3.0–4.4%	Total content	[60]
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	DLLME (disperser solvent: acetone; extraction solvent: trichloromethane)	UHPLC-MS/MS	LOD: 0.1–0.2 ng mL^{-1} LOQ: 0.3–0.6 ng mL^{-1} R: 88–104% RSD: 0.5–22.5%	Total and free forms content	[61]
BP-3, 4-MBC, HS, OC	ASE & SPE	GC-MS/MS	LOD: 0.47–0.59 pg mL^{-1} LOQ: no data R: 70.5–110.7% RSD: <5.04%	Total and free forms content	[62]
BMDBM, CDAA, EHS, 5-OH-EHS, OC	LLE (solvent: actonitrile)	LC-LC-MS/MS (ESI); type of column: RP-18 ADS;	LOD: 0.1–1.5 µg L^{-1} LOQ: 0.2–4.1 µg L^{-1} R: 94.2–113.6% RSD: 2.6–16.5%	Total content	[63]
5OH-EHS, 5oxo-EHS, 5cx-EPS	Online SPE (TurboFlow Phenyl)	HPLC-MS/MS (ESI); type of column: C_{18}; mobile phase: gradient acetonitryle/water/0.05% acetic acid	LOD: no data LOQ: 0.01–0.15 µg L^{-1} R: 96–106% RSD: 1.2–2.4%	Total and free forms content	[64]
BP-3	Online SPE (RP_{18})	HPLC-MS/MS (ESI); type of column: XDB-C_{18}; mobile phase: gradient methanol/water	LOD: 0.16 µg L^{-1} LOQ: no data R: 101% RSD: 5%	Total and free forms content	[65]
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	LLE (solvent: ethyl *tert*-butyl ether/ethyl acetate (5:1; *v*:*v*))	UHPLC-TQMS (ESI^-); type of column: C_{18}; column temp.: 30 °C; mobile phase: water/acetonitrile	LOD: 0.01–0.2 ng mL^{-1} LOQ: no data R: 90.7–110.1% RSD: 6.9–14.2%	Total and free forms content	[66]
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	VADLLME (disperser solvent: 2-propanol; extraction solvent: dichloromethane)	LC-MS/MS; type of column: C_{18}; column temp.: 23 °C; mobile phase: water/methanol	LOD: 0.02–0.03 ng mL^{-1} LOQ: 0.05–0.4 ng mL^{-1} R: no data RSD: 1.2–12%	Total content	[67]
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	AALLME (extraction solvent: 1,2-dichloroethane)	LC-MS/MS (ESI); type of column: C_{18}; column temp.: 40 °C; mobile phase: water/methanol	LOD: 0.02–0.06 ng mL^{-1} LOQ: 0.05–0.20 ng mL^{-1} R: no data RSD: <15%	Total content	[68]
PABA, 4-AHA, 4-AMB, 4-OCH_3-AHA	LLE & SPE (solvent: ethyl acetate; C_{18})	HPLC-ECD; type of column: C_{18}; mobile phase: methanol/phosphate buffer (pH 5.5) (20:80; *v*/*v*)	LOD: no data LOQ: 0.04–0.18 ng mL^{-1} R: 96–99% RSD: 0.2–3.8%	Total content	[69]
BP-1, BP-3	SPE (C_8)	HPLC-UV; type of column: C_{18}; mobile phase: acetonitryle/water	LOD: 2–40 ng mL^{-1} LOQ: no data R: no data RSD: 6.6–13%	Total and free form content	[70]

Table 3. *Cont.*

UV Filters	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
PMDSA	Online SPE	SIA-FL	LOD: 12 ng mL^{-1} LOQ: no data R: no data RSD: 2–13%	Free forms	[71]
PEG-25 PABA	SPE (C_{18})	LC-FL; mobile phase: dimethylfuran	LOD: 2.6 ng mL^{-1} LOQ: no data R: 91–100% RSD: 3–10%	Total content	[72]
BP-4, 4-DHB, BP-2, BP-1, BP-8, BZ	FPSE	HPLC-PDA; type of column: C_{18}; mobile phase: methanol/phosphate buffer (pH 3) (45:55; *v*/*v*)	LOD: 0.03 µg mL^{-1} LOQ: 0.1 µg mL^{-1} R: no data RSD: 2.3–14.4%	Total content	[73]
EDP	In-vial MMLLE (hydrophobic PTFE membranes)	GC-MS; type of column: SPB-5; oven temp.: 60 °C/1.5 min—30 °C/1 min to 275 °C/20 min	LOD: no data LOQ: 0.11 µg L^{-1} R: no data RSD: 7.4%	Total content	[74]
BP-3, BP-4	SI SPE (C_{18} and diethylaminopropyl)	LC/UV; type of column: RP_{18}; mobile phase: ethanol/acetate buffer/1% acetic acid	LOD: 30–60 ng mL^{-1} LOQ: no data R: no data RSD: 6–13%	Free forms	[75]
BP-1, BP-2, BP-8, 4-OH-BP	MEPS (C_{18})	LC-MS/MS; mobile phase: water/methanol	LOD: 0.005–0.03 ng mL^{-1} LOQ: 0.02–0.10 ng mL^{-1} R: 18–118% RSD: 1–16%	Total and free forms content	[78]
BP-1, BP-3, BP-8	SPME (Carbowax/DVB)	GC-MS; type of column: DB5-MS; Oven temp.: 50 °C/0.1 min—30 °C/1 min to 150 °C—18 °C/1 min to 250 °C/12 min	LOD: 5–10 ng mL^{-1} LOQ: no data R: no data RSD: 5–8%	Total content	[79]
BP, BP-1, BP-3, 4-OH-BP	BAµE	HPLC–DAD; type of column: Sea-18; mobile phase: methanol/water (75:25; *v*/*v*)	LOD(P2): <1.0 µg L^{-1} LOQ(P2): <0.3 µg L^{-1} LOD(AC4): <1.3 µg L^{-1} LOQ(AC4): <0.4 µg L^{-1}	Total content	[76]
OMC, BP-3, OC, OS, HS	DLLME (disperser solvent: carbon tetrachloride; extraction solvent: acetonitrile)	HPLC-DAD; type of column: C_{18}; mobile phase: isocratic water/methanol/acetonitrile (8:42:50; *v*/*v*/*v*)	LOD: no data LOQ: 3–45 ng mL^{-1} R: 86.9–97.3% RSD: 0.1–6.4%	Total content	[77]
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	Microextraction using a monolithic stirring extraction unit (150 min; 1100 rpm)	UPLC-DAD; mobile phase: acetonitrile/water	LOD: 1–10 µg L^{-1} LOQ: 5–20 µg L^{-1} R: 71–114 % RSD: 5.6–9.1%	Total content	[80]

The liquid–liquid extraction is a time-consuming technique, which requires large volumes of organic solvents, and is not automated. It uses different types of organic solvents such as ethyl acetate, a mixture of methyl tert-butyl ether: ethyl acetate, ethanol, methanol, and acetonitrile. The solid-phase extraction is used in manual mode or an online configuration or in commercially available automated workstations. Octadecyl silica sorbents (C18) are widely used for UV filter analysis using SPE in manual mode; divinylbenzene/N-vinylpyrrolidone copolymer (HLB) is an alternative option in this regard. The microextraction techniques are based on the equilibrium processes. Additionally, solid-phase microextraction (SPME) is based on the division of the analyte between the urine sample and a sorbent such as carbowax-DVB fibre. Stir-bar sorptive extraction (SBSE) uses the polymer coating of polydimethylsiloxane as a sorbent. Another microextraction technique is the microextraction by packed sorbent (MEPS), which uses the C18 sorbent to extract analytes. Yet another technique is the dispersive liquid–liquid microextraction (DLLME), which uses solvents (dispersing—acetone and extracting—trichloromethane). Different microextraction methods include hollow-fibre liquid-phase microextraction (HFLPME),

based on the use of polypropylene porous hollow fibre, air-assisted liquid-liquid microextraction (AALLME), bar adsorptive microextraction (BAμE), single-drop microextraction (SDME), and vortex-assisted dispersive liquid–liquid microextraction (VADLLME). The final steps are attaining lyophilisation and redissolution of the residue in the solvent.

When examining plasma or serum, blood must undergo additional treatment to isolate them (Table 4). Plasma also includes large proteins such as albumin or immunoglobulin. Such treatment consists in the centrifugation of fresh blood with the addition of an anticoagulant. Serum, however, is prepared by centrifuging blood samples without anticoagulant. To determinate the total compound content, the hydrolysis step must be performed with either acid [81] or an enzyme solution [82–85]. In the case of blood, serum, or plasma samples, protein precipitation is commonly used to reduce matrix interferences. This is performed by mixing the sample with such organic solvents as acetonitrile [60,63,86], methanol [73,81], acetone [83], or formic acid [84,85]. Proteins are denatured, precipitated, and separated through centrifugation.

Table 4. Published studies on UV filters determination in blood, plasma, and serum.

UV Filters	Matrix	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
BP-3, BP-1, BP-8	Serum	DLLME (disperser solvent: acetone: extraction solvent: chloroform)	LC-MS/MS (ESI^+); type of column: C_{18}; mobile phase: gradient methanol/water/0.1% formic acid	LOD: 7–8 μg L^{-1} LOQ: 22–28 μg L^{-1} R: 77–104% RSD: 8–9%	Total content	[45]
BP-3, OMC, OS, HS	Plasma	LLE (solvent: acetonitrile)	HPLC-DAD; type of column: C_{18}; mobile phase: gradient methanol/water (75:25; *v*/*v*)	LOD: 0.03–0.2 μg mL^{-1} LOQ: 0.1–0.4 μg mL^{-1} R: 90.8–103.8% RSD: 2.1–4.4%	Total content	[60]
BP-3, OMC, OS, HS	Bovine serum albumin	LLE (solvent; acetonitrile)	HPLC-DAD; type of column: C_{18}; mobile phase: gradient methanol/ water (75:25; *v*/*v*)	LOD: 0.03–0.2 μg mL^{-1} LOQ: 0.1–0.4 μg mL^{-1} R: 97.9–102.3% RSD: 1.2–3.3%	Total content	[60]
BP-1, BP-2, BP-3, BP-6, BP-8, 4-OH-BP	Menstrual blood	DLLME (disperser solvent: acetone; extraction solvent: trichloromethane)	UHPLC-MS/MS (ESI); type of column: C_{18};	LOD: 0.2–0.3 ng mL^{-1} LOQ: no data R: no data RSD: 0.28–1.59%	Total and free forms content	[82]
BP-1, BP-2, BP-3, BP-6, BP-8, 4-OH-BP	Serum	DLLME (disperser solvent: acetone; extraction solvent: trichloromethane)	UPLC-MS/MS (ESI^+); type of column: C_{18}; mobile phase: gradient 0.1% ammoniacal aq/0.1% ammonia in methanol	LOD: 0.1–0.3 ng mL^{-1} LOQ: 0.4–0.9 ng mL^{-1} R: 97–106% RSD: 1.9–13.7%	Total and free forms content	[83]
BP-3	Serum	Online SPE	HPLC-MS/MS ($APPI^-$)	LOD: 0.5 ng mL^{-1} LOQ: no data R: 96% RSD: 7.7–8.7%	Total content	[84,85]
OC, BMDBM, CDAA	Plasma	LLE (solvent: acetonitrile)	LC-LC-MS/MS (ESI); type of column: C_{18}; mobile phase: methanol/water	LOD: 1.1–6.5 μg L^{-1} LOQ: 3.5–20.7 μg L^{-1} R: 89.0–112.8% RSD: 3.0–4.9%	Total content	[63]
BP-3	Plasma	LLE (solvent: acetonitrile)	UHPLC-DAD; type of column: C_{18}; mobile phase: acetonitrile/water	LOD: no data LOQ: no data R: 94–99% RSD: 2.3–4.6%	Total content	[86]
BP-4, 4-DHB, BP-2, BP-1, BP-8, BZ	Whole blood	FPSE	HPLC-PDA; type of column: C_{18}; mobile phase: methanol/phosphate buffer (pH 3) (45:55; *v*/*v*)	LOD: 0.03 μg mL^{-1} LOQ: 0.1 μg mL^{-1} R: no data RSD: 0.4–10.8%	Total content	[73]

Table 4. *Cont.*

UV Filters	Matrix	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
BP-4, 4-DHB, BP-2, BP-1, BP-8, BZ	Plasma	FPSE	HPLC-PDA; type of column: C_{18}; mobile phase: methanol/phosphate buffer (pH 3) (45:55; *v*/*v*)	LOD: 0.03 μg mL^{-1} LOQ: 0.1 μg mL^{-1} R: no data RSD: 3.6–11.1%	Total content	[73]
BP-3, BP-1, 4-OH-BP, BP-8, 4-DHB, BP-2, BP-4, BMDBM	Umbilical cord blood	LLE (solvent: MTBE)	LC-MS/MS (ESI^+; ESI^-); type of column: R_{18}; mobile phase: methanol/water	LOD: 0.05–0.42 ng mL^{-1} LOQ: 0.18–1.39 ng mL^{-1} R: 14.3–146.4% RSD: 0.5–33.8%	Total content	[81]
BP, 4-MBP	Plasma	LLE-SPE (solvent: MTBE; Oasis Prime-HLB)	HPLC-MS/MS (ESI); type of column: C_{18}; mobile phase: 0.1% formic acid in water/0.1% formic acid in methanol	LOD: 0.8–2 pg mL^{1} LOQ: 3.5–7 pg mL^{-1} R: 87–97% RSD: 3.1–9.1%	Total content	[87]

Table 5. Published studies on UV filters determination in semen, saliva, milk, nail, and placental tissue.

UV Filters	Matrix	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
BP-1, BP-3, BP-8, THB	Semen	SPE (C_{18})	LC-MS/MS (ESI^+); type of column: Mediterranean SEA 18; mobile phase: gradient mobile phase: 0.1% formic acid in water/0.1% formic acid in methanol	LOD: 0.03–0.04 ng mL^{-1} LOQ: 0.08–0.13 ng mL^{-1} R: 98–115% RSD: no data	Total content	[56]
BP-3, OMC, OS, HS	Epidermal membranes	LLE (solvent: acetonitrile)	HPLC-DAD; type of column: C_{18}; mobile phase: gradient methanol/water (75:25; *v*/*v*)	LOD: 0.03–0.2 μg mL^{-1} LOQ: 0.1–0.4 μg mL^{-1} R: 98.5–99.5% RSD: 1.8–3.2%	Total content	[60]
OC, 3-BC, 4MBC, OMC, EDP, BP-1, BP-3, BP-6, BP-8, 4-OH-BP	Milk	QuEChERS Extraction; SALLE & d-SPE (sorbent: polysecondary amine and magnesium sulphate)	UHPLC-MS/MS (API); type of column: C_{18}; mobile phase: gradient acetonirile/water/0.1% formic acid	LOD: 0.1–0.2 ng mL^{1} LOQ: 0.4–0.6 ng mL^{-1} R: 87–112% RSD: 8–14%	Total content	[88]
BP-3	Breast milk	Online SPE (RP_{18})	HPLC-MS/MS ($APCI^-$); type of column: RP_{18}; mobile phase: gradient methanol/water	LOD: 0.51 ng mL^{-1} LOQ: no data R: 94.7% RSD: 12.7–18%	Total and free forms content	[89]
BP-1, BP-3, 4-OH-BP, 4DHB, 4MBC, ODPABA, EtPABA, TBHPBT	Breast milk	Online TFC	HPLC-MS/MS (ESI); type of column: Cyclone and C_{18}; mobile phase: gradient methanol/water/0.1% formic acid	LOD: 0.1–1.5 ng g^{-1} LOQ: 0.3–5.1 ng g^{-1} R: no data RSD: 1–12%	Total content	[90]
BP-3	Milk	Online SPE (RP_{18})	HPLC-MS/MS ($APCI^-$); type of column: RP_{18}; mobile phase: methanol/water	LOD: 0.4 ng mL^{-1} LOQ: no data R: 102% RSD: 8.8–12%	Total and free forms content	[91]
BP-1, BP-3, BP-6, BP-8, 4-OH-BP	Breast milk	USAD-SPE (15 min of sonification; sorbents: C_{18}, polysecondary amine and magnesium sulphate)	UHPLC-MS/MS (ESI^+); type of column: C_{18}; mobile phase: gradient aqueous ammonium formate solution (pH 9)/0.025% ammonia in MeOH	LOD: 0.1–0.2 ng mL^{-1} LOQ: 0.3–0.6 ng mL^{-1} R: 90.9–109.5% RSD: 2.0–12.3%	Total content	[92]
BP-1, BP-2, BP-3, BP-6, BP-8, 4-OH-BP, THB, AVB	Nail	MAE (20 min, 1000 W of power)	UHPLC-MS/MS (ESI^+); type of column: C_{18}; mobile phase: gradient methanol/water/0.1% formic acid	LOD: 0.2–1.5 ng g^{-1} LOQ: 1.0–5.0 ng g^{-1} R: 90.2–112.2% RSD: 0.8–12.3%	Total content	[93]
BP-1, BP-2, BP-3, BP-6, BP-8, 4-OH-BP	Placental tissue	MSPD (solvent: ethyl acetate)	UHPLC-MS/MS (ESI); type of column: C_{18}; mobile phase: gradient 0.1% ammoniacal aq solution/0.1% ammonia in methanol	LOD: 0.1 ng g^{-1} LOQ: 0.2–0.4 ng g^{-1} R: 95–106% RSD: 4.5–11.8%	Free forms	[94]
BP-1, BP-2, BP-3, BP-4, 4-OH-BP	Placental tissue	SLE (solvent: ethyl acetate)	LC-MS/MS (ESI^-); type of column: RP_{18}; mobile phase: gradient methanol/water	LOD: 0.02–0.36 ng mL^{-1} LOQ: 0.05–1.20 ng mL^{-1} R: 72–110% RSD: 4–40%	Total content	[95]

Table 5. *Cont.*

UV Filters	Matrix	Extraction Technique	Analytical Technique	Analytical Performance	Comments	Ref.
BP-1, BP-2, BP-3, BP-8, 4-OH-BP	Saliva	DLLME (disperser solvent: acetone; extraction solvent: trichloromethane)	LC-MS/MS; type of column: C_{18}; mobile phase: gradient methanol/water	LOD: 0.01–0.15 ng mL^{-1} LOQ: 0.05–0.40 ng mL^{-1} R: no data RSD: 1–19%	Total content	[96]
EDP, 3-BC, MBC, OMC, OC, BP-1, BP-3, BP-6, BP-8, 4-OH-BP	Placenta tissue	UAE (disperser solvent: methanol; extraction solvent: anisole; 3 min of sonification)	UHPLC-MS/MS; type of column: C_{18}; mobile phase: gradient acetonitrile/0.25% formic acid aq	LOD: 0.05–0.2 µg kg^{-1} LOQ: 0.15–0.5 µg kg^{-1} R: 90–112% RSD: 3–15%	Total content	[97]

The most popular extraction technique in the case of plasma, serum, or blood samples is liquid–liquid extraction with the use of such organic solvents as acetonitrile [60,63,86], as well as a methyl tert-butyl ether [81,87] (Table 4). Another technique is dispersive liquid–liquid microextraction (DLLME) with the use of acetone as the disperser solvent and trichloromethane as the extraction solvent [82,83] or acetone as the disperser solvent and chloroform as the extraction solvent [45]. Solid-phase extraction with the C18 sorbent [84,85] and fabric phase sorptive extraction (FPSE) [73] have also been employed.

In the case of milk, semen, and silva samples, determination takes place in the same way as for urine and plasma samples, and as such, the first step is the acid or enzymatic hydrolysis [69,88–90]. Afterwards, acetonitrile [88,90], formic acid [56], isopropanol [89], or methanol [91] is added to precipitate proteins. Finally, in the case of other biological samples such as placenta, nail, or epidermal membrane tissue, homogenisation takes place as well. The samples are shaken and mixed to enable tissue break up (Table 5).

The extraction techniques used in the determination of UV filters in milk, semen, and tissue samples are the same as in the case of urine, i.e., solid-phase extraction, in manual mode [56] and online configuration [89,91]; the ultrasound-assisted dispersive solid-phase extraction (USAD-SPE) is employed as well [92]. Microwave-assisted digestion/extraction (MAE) [93], matrix solid-phase dispersion (MSPD) [94], solid–liquid extraction (SLE) [95], dispersive liquid–liquid microextraction [96], and ultrasound-assisted extraction (UAE) [97] have also been applied for this purpose (Table 5).

3.2. Analytical Techniques

Even if an exhaustive initial sample treatment is performed to eliminate possible interfering compounds from the sample, an adequate analytical separation technique must still be selected to improve analyte determination. Tables 3–5 present the most used analytical techniques for the detection and quantification of UV filters in biological samples. Liquid chromatography and gas chromatography coupled with MS or MS/MS is the most frequent choice. The choice of either GC or LC is mainly based on the physicochemical properties of the target compounds. GC is usually employed to determinate volatile analytes, whereas LC is applied to quantify both more polar and less volatile compounds.

Liquid chromatography has been used most widely for the determination of UV filters in biological samples. LC coupled with mass spectrometry detectors in tandem is the preferable option. Various ionisation sources have also been used. The most frequently used ionisation mode has been electrospray ionisation (ESI) [45,46,51,55,59,63–65,81–83,87,90,92,94–97]. Moreover, it was found that ESI^+ has better efficiency than ESI^- [56]. It is a soft ionisation technique suitable for polar and mildly non-polar compounds. Nevertheless, since ion suppression or improvement in the complex matrix may occur, atmospheric pressure chemical ionisation (APCI) [47–49,75,77] and atmospheric pressure photoionisation (APPI) [84,85] have also been used. In all mentioned cases, the determination was carried by multiple reaction monitoring (MRM) mode of the most intense transition, with another one employed to confirm the presence of UV filters in biological matrices at very low concentration levels. Yet another type of detector coupled to liquid chromatography is based on UV/Vis spectroscopy. It is often used due to the fact that UV filters exhibit a high absorbance in the UV range of the electromagnetic spectrum [44,52,60,70,75–77,80,86]. Liquid chromatography coupled with a fluorometric detector has been scarcely used because most UV filters do not

exhibit fluorescence properties. LC-FL was only used twice—in determining PBSA [71], as well as PEG-25 and PABA [72] in urine samples.

While gas chromatography has been used less often, in most cases it is coupled with mass spectrometry with electron impact [53,54,62,74,79]. In the case of UV filters, a derivatisation step is required before the GC analysis. UV filters have been typically derivatized by using such silylating reagents as N,O-Bis (trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BSTFA-TMCS) [62] or N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) [54].

Lastly, despite comprehensive sample preparation and the use of carefully select analytical techniques, it must be noted that final results may sometimes be affected by the "matrix effect." This phenomenon may impact quantitative recoveries when using external calibration. As such, it may cause differences in the behaviour of the analytes with the accompanying matrix compounds that one can use to enhance or decrease the signal (e.g., ion suppression in the mass spectrum) or affect the extraction efficiency when the extraction technique is used. This negative effect has been adjusted for by using a matrix-matched calibration (the use of the same matrix without analytes to prepare the standard calibration solutions). In other cases, the standard addition calibration method or an isotopic internal standard was used.

3.3. Accuracy and Sensitivity

Tables 3–5 show information about achieved results for different analytical methods used for the determination of UV filters in biological samples.

The analytical methods presented in it resulted in recoveries enabling exhaustive quantification of the target UV filters in the biological matrices, using external or matrix-matched or standard addition calibration. Thus, in the case of urine samples, the greatest recoveries have been achieved for BP-2 (118%) using microextraction by packed sorbent [78] and for EHS (113%) using liquid–liquid extraction [63]. In the case of blood, plasma, and serum samples, the best recoveries have been obtained for BP-1 (146.4%) using liquid–liquid extraction [81]. In milk samples, the highest-level recoveries have been achieved for BP-3 (112%) by using salt-assisted liquid–liquid extraction coupled with dispersive solid-phase extraction [88]. The recoveries in the case of the determination of OMC in placenta tissue by using ultrasound-assisted extraction amounted up to 112% [97].

In terms of sensitivity, the published methods (Tables 3–5) enable the determination of UV filters in the low pg mL^{-1} range.

In the urine samples, the lowest limit of detection (LOD) has been achieved for BP-3 (5 pg mL^{-1}) using hollow-fibre liquid-phase microextraction [42]. The LOD for BP-3, 4-MBC, OC, and HS (0.47–0.59 pg mL^{-1}) was obtained by using accelerated solvent extraction coupled with solid-phase extraction [62]. In the plasma sample, the LOD was at a level of 0.8 pg mL^{-1} for BP; it was determined using liquid–liquid extraction in conjunction with solid-phase extraction [87].

In the milk sample, the best LOD has been achieved for BP-6 and BP-1 (0.1 ng mL^{-1}) using salt-assisted liquid–liquid extraction coupled with dispersive solid-phase extraction [69]. In the determination of 4-OH-BP in the tissue sample, the LOD of 0.02–10 ng mL^{-1} has been obtained using solid–liquid extraction [95].

The low levels achieved in the determination of UV filters in biological samples have been influenced by the use of sensitive analytical techniques (e.g., MS/MS), as well as such enrichment techniques as LLE, SPE, MALLE, SPME, SBSE, SDME, HF-LPME, and MALLME.

4. Conclusions

Organic UV filters are a family of cosmetic ingredients most widely used in a common variety of cosmetic products to protect consumers from UV solar radiation. Since compounds belonging to this group can be metabolised, excreted, and/or bioaccumulated,

UV filters may be harmful to the human body. This has made analysing UV filters both in cosmetics products and biological samples a necessity.

Liquid chromatography with MS or UV detection is the dominant method for the determination of UV filters. The large majority of published works used conventional C18 or C8 separation columns. Due to the low level of UV filters in the biological samples (e.g., urine, blood, milk), it is necessary to perform the extraction and clean-up steps before the determination procedure to improve the detection limits. LLE and SPE are the most widely used sample preparation and enrichment methods among all those used. However, these conventional techniques present some drawbacks, such as the consumption of large volumes of sample and often toxic organic solvents, but they are time consuming. Nonetheless, such modern microextraction techniques as MEPS, SPME, SBSE, or DLLME are used as well. However, they are only used in 25% of analytical procedures. Due to the trends of modern analytical techniques towards "Green Analytical Chemistry," they should in the future replace the classic methods of preparing samples for research. This is because of their many advantages, i.e., time-consuming and labour intensity, and above all because they are solvent-free methods.

This review paid special attention to the analytical performance, e.g., limits of detection, accuracy, and repeatability for developed and validated analytical methods. Organic UV filters have been determined to be prevalent in all kinds of biological matrices and are associated with specific markers connected to metabolism, physiological development, and harmful effects in the human body.

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Abbreviations

$[C_6MIM][PF_6]$:	hexyl-3-methylimidazolium hexafluorophosphate
2-OH-BP:	2-hydroxybenzophenone
3-BC:	3-benzophenone camphor
4-AHA:	p-aminohippuric acid
4-AMB:	p-acetamidobenzoic acid
4-DHB:	4,4-dihydroxybenzophenone
4-MBC:	3-(4-methylbenzylidene)-camphor
4-OCH_3-AHA:	p-acetamidohippuric acid
4-OH-BP:	4-hydroxybenzophenone
5cx-EPS:	5-(((2-hydroxybenzoyl)oxy)methyl)heptanoic acid
5-OH-EHS:	5-hydroxy-2-ethylhexyl salicylate
5oxo-EHS:	2-ethyl-5-oxohexyl 2-hydroxybenzoate
AALME:	air-assisted liquid–liquid microextraction
Ac:	Acetone
APCI:	atmosphere pressure chemical ionisation
API:	atmosphere pressure ionisation
APPI:	atmosphere pressure photoionisation
ASE:	accelerated solvent extraction
BMDBM:	butyl methoxydibenzoylmethane/avobenzene
BAμE:	bar adsorptive microextraction

BC:	benzyl cinnamate
BDM:	butyl methoxydibenzoylmethane
EMT:	bis-ethylhexyloxyphenol methoxyphenyl triazine
BP:	Benzophenone
BP-1:	2,4-dihydroxybenzophenone
BP-10:	2-hydroxy-4-methoxy-4′-methylbenzophenone
BP-12:	(2-hydroxy-4-octoxy-phenyl)-phenyl-methanone
BP-2:	2,2′,4,4′-tetrahydroxybenzophenone
BP-3:	2-hydroxy-4-methoxybenzophenone
BP-4:	2-hydroxy-4-methoxybenzophenone-5-sulphonic acid
BP-6:	2,2′-dihydroxy-4,4′-dimethoxybenzophenone
BP-7:	5-chloro-2- hydroxybenzophenone
BP-8:	2,2′-dihydroxy-4-methoxybenzophenone
BP-OH:	Benzhydrol
BS:	benzyl salicate
BZT:	Benzotriazole
C18:	Octadecyl
CDAA:	2-cyano-3,3-diphenyl acrylic acid
CPE:	cloud point extraction
DAD:	diode-array detection
DART-MS:	direct-analysis-in-real-time mass spectrometry
DBT:	diethylhexyl butamino triazone
DCM:	Dichloromethane
DEA:	Diethylaminopropyl
DHHB:	diethyloamino hydroxybenzoyl hexyl benzoate
DLLME:	dispersive liquid–liquid microextraction
DMF:	n,n-dimethylformamide
DTS:	drometrizole trisiloxane
DS:	Densitometry
d-SPE:	dispersive solid-phase extraction
EA:	ethyl acetate
ECD:	electron captur detector
EDP:	2-ethylhexyl 4-(n,n-dimethylamino)benzoate
EHC:	ethylhexyl cinnamate
EHS:	2-ethylhexyl salicylate
EI:	electron impact
EMC:	ethylhexyl methoxycinnamate
EHS:	ethylhexyl salicylate
ESI:	electrospray ionisation
ET:	ethylhexyl triazone
EtOH:	Ethanol
EtPABA:	ethyl p-aminobenzoic acid
FL:	Fluorescence
FPSE:	fabric phase sorptive extraction
GC:	gas chromatography
HFLPME:	hollow-fiber liquid-phase microextraction
HPLC:	high-performance liquid chromatography
HS:	salicylic acid 3,3,5-trimethcyclohexyl ester
HTLC:	high-temperature liquid chromatographic
IMC:	isoamyl p-methoxycinnamate
LC:	liquid chromatography
LD:	liquid desorption
LLE:	liquid–liquid extraction
LOD:	limit of detection
$\log_{Ko/w}$:	log octanol/water partition coefficient
LOQ:	limit of quantification
LTP-MS:	low temperature plasma ionisation mass spectrometry

MA:	menthyl anthranilate
MAE:	microwave-assisted extraction
MBBT:	methylene bis-benzotriazolyl tetramethyl butyl phenol
MBC:	4-methylbenzylidene camphor
MBP:	methylene bis-benzotriazoyl tetramethylbutylphenol
MeCN:	Acetonitrile
MEKC:	micellar electrokinetic capillary chromatography
MeOH:	Methanol
MEPS:	microextraction by packed sorbent
MMLLE:	microporous membrane liquid–liquid extraction
MS/MS:	tandem mass spectrometry
MS:	mass spectrometry
MSPD:	matrix solid phase dispersion
MTBE:	methyl tert-butyl ether
NaCl:	sodium chloride
OC:	4-methylbenzilidene camphor/octocrylane
ODP:	octyl dimethyl PABA
ODPABA:	2-ethylhexyl 4-(dimethylamino)benzoate
OMC:	2-ethylhexyl p-methoxycinnamate
OS:	2-ethylhexylsalicylate
PABA:	p-aminobenzoic acid
PMDSA:	2-phenylbenzimidazole-5-sulphonic acid
PDA:	photodiode-array detection
PEG-25 PABA:	polyethylene glycol 25 paminobenzoic acid
PHBA:	4-hydroxy benzoic acid
PLE:	pressurized liquid extraction
p_{Ka}	acid dissociation constant
PSA:	primary-secondary amine
QuEChERSExtraction:	Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction
R:	Recovery
RSD:	relative standard deviation
SALLE:	salt-assisted liquid–liquid extraction
SBSE:	stir bar sorptive extraction
SDME:	single-drop microextraction
SFC:	supercritical fluid chromatography
SIA:	sequential injection analysis
SI SPE:	sequential injection solid-phase extraction
SLE:	solid–liquid extraction
SPE:	solid-phase extraction
SPME:	solid-phase microextraction
SWV:	squarewave voltammetry
TBHPBT:	2-(5-tert-butyl-2-hydroxyphenyl)benzotriazole
TCM:	trichloroamine
TFA:	trifluoroacetic acid
TFC:	turbulent flow chromatography
THB:	2,3,4-trihydroxybenzophenone
TLC:	thin-layer chromatography
UAE:	ultrasound-assisted extraction
UHPLC:	ultra-high-performance liquid chromatography
UHPSFC:	ultra-high performance supercritical fluid chromatography
UPLC:	ultra-performance liquid chromatography
USAD-SPE:	ultrasound-assisted dispersive solid phase extraction
UV/Vis:	ultraviolet/visible spectrometry
VADLLME:	vortex-assisted dispersive liquid–liquid microextraction

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Determination of Benzophenones in Water and Cosmetics Samples: A Comparison of Solid-Phase Extraction and Microextraction by Packed Sorbent Methods

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Abstract: Benzophenones (BPs) are extensively used in a wide variety of cosmetic products and other materials (e.g., textiles or plastics) to avoid damaging effects of UV radiation. In the present work, we compared two extraction methods for the determination of BPs, namely, 2,4-dihydroxybenzophenone (BP-1), 2-hydroxy-4-methoxybenzophenone (BP-3) and 2,2-dihydroxy-4-methoxybenzophenone (BP-8), in water and cosmetics samples. The following extraction methods were used for the research: solid-phase extraction (SPE) and microextraction by packed sorbent (MEPS), whereas analysis was performed by gas chromatography with mass spectrometric detection. A comparison between the methods indicates that the MEPS technique(s) can be reliably used for analysis of BPs (sunscreen residue) in water samples and cosmetic samples with satisfactory results. This microextraction technique is cheap, easy, quick to implement, and consumes small amounts of solvents. On the other hand, the main advantage of the SPE method are low detection limits for the determination of BPs in water samples, i.e., from 0.034 to 0.067 μg L^{-1}, while, for the MEPS method, LODs were at the level of 1.8–3.2 μg L^{-1}. For both methods, the recoveries of BPs were 96–107% and 44–70% for water and cosmetics samples, respectively. The presented methods are suitable for use in cosmetics quality control and environmental pollution assessment.

Keywords: benzophenones; analysis of cosmetics; microextraction by packed sorbent; solid-phase extraction; water analysis

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1. Introduction

The ultraviolet (UV) filters, especially benzophenones (BPs), are most used in sunscreen products, cosmetics, lipsticks, hair sprays, hair dyes, shampoos, and other personal care products. Moreover, they can be found as additives in textiles, plastics, paints, car polishes, etc.

Benzophenone-3 (2-hydroxy-4-methoxybenzophenone (BP-3)), benzophenone-1 (2,4-dihydroxybenzophenone (BP-1)), and benzophenone-8 (2,2-dihydroxy-4-methoxybenzophe none (BP-8)) are very often used in sunscreens to protect human skin from ultraviolet radiation. Due to the high effectiveness of benzophenones and their appropriate properties, such as absorption or reflection of UV radiation in a wide range, they are chemically stable, e.g., they do not decompose in the cosmetic, under the influence of the sun or under the influence of other factors, do not evaporate after application, do not they cause staining of the skin, they do not smell, they are approved for use. Its recommended maximum content to 10% (*w*/*w*) in cosmetics has been formulated by appropriate legislation in many countries (Australia, Europe, China, and the Mercosur) [1–4].

These compounds can enter the aqueous environment directly or indirectly, for example, as a result of swimming and bathing in lakes and rivers, from showering, washing, and via wastewater treatment plants, by virtue of which they are ever-more present in

environmental waters [4–6]. They are photostable, lipophilic, and potentially bioaccumulative compounds. The relatively high log octanol/water partition coefficient (log Kow > 3) value of BPs suggests its slow biodegradation and tendency to adsorb the suspended solids and sediments [4,7]. Nowadays, there is evidence to support the fact that BP-3 is absorbed through the skin and can bioaccumulate both in wildlife and humans [4]. Some of these compounds have been found in fish, urine, and breast milk [5,8–10]. In the last years, different toxicological studies conducted in vitro or in vivo in animals suggested that some of UV filters show significant estrogenic and/or antiandrogenic activity [4].

Among BPs, BP-3 is one of the most often detected in surface water from bathing areas [7]. When BP-3 is applied on the skin, it is partially absorbed by the human body and excreted as more polar metabolites, such as BP-1 and BP-8. They are also used as UV absorbers to protect goods against UV radiation. BP-1 is also the main metabolite of BP-3, identified in human urine. BP-8 is considered as a genotoxic compound [1,7]. Moreover, they are prone to evolve into halogenated by-products when mixed with chlorine ions [1,11,12]. The presence of BPs in the environment and their content in cosmetics should be monitored.

There are no official analytical methods for the determination of BPs in cosmetic products. In our previous literature report [13], we presented the methods used to measure BPs in water, urine, tissues, and cosmetics. To summarize, according to the literature data, the methods such as supercritical fluid extraction (SFE) in combination with capillary electrophoresis (CZE) and high-performance liquid chromatography (HPLC) [14] or polarographic method [15] are applied for the determination of BPs in cosmetic products. In the case of cosmetic samples, the method of gas chromatography coupled with MS is used very rarely [13]. Consequently, there is a great interest in the development of sensitive and selective analytical methods to ensure consumer health and the control of environmental pollution.

Gas chromatography and high-performance liquid chromatography in combination with mass spectrometry (GC-MS, HPLC-MS) is the most common method and it allows the accurate determination of UV filters in water samples [15]. Content of BPs in the environmental water samples is in trace amounts so that a preconcentration step must be carried out prior to their chromatography analysis.

For this purpose, the most common sample preparation techniques are used, such as liquid–liquid extraction (LLE) [16] and solid-phase extraction (SPE) [1,17,18], as well as microextraction techniques such as solid-phase microextraction (SPME) [19–22], single drop microextraction [23], dispersive liquid–liquid microextraction (DLLME) [6,24–26], stir bar sorptive extraction (SBSE) [27–31], microextraction by packed sorbent (MEPS) [32,33], and stir bar sorptive-dispersive microextraction (SBSDμE) [34]. The dispersive micro solid-phase extraction ((DI)μ-SPE) [35], fabric phase sorptive extraction (FPSE) [36], and pressurized liquid extraction (PLE) [37] are used also.

In the case of LLE and SPE, the main disadvantages are that it is time-intensive, uses large amounts of potentially toxic and expensive organic solvents, and requires high sample manipulation. Therefore nowadays, the so-called microextraction techniques play an important role in the sample preparation of environmental water for analysis, while microextraction methods such as SPME and SBSE use expensive, easy to damage materials and usually have carry over effects. However, they also have many advantages. SPME is fast, sensitive, solvent-free, and simple, whereas SBSE with thermal desorption is characterized by a very low limit of detection, while MEPS is a relatively new miniaturized SPE technique where the sorbent bed (1–4 mg) is integrated into the liquid handling syringe (100–250 μL). First, this technique is simple to operate, fast, inexpensive, precise, sensitive, environmentally friendly, and almost solventless [8]. Additionally, MEPS can be used for various types of matrices. Therefore, we decided to check the suitability of this method for the determination of BPs in cosmetics samples.

We applied MEPS and SPE techniques to compare both methods, especially in terms to determine BPs in complex matrices such as different cosmetics samples. To our knowledge

this is the first paper reporting application of these methods prior to analysis by gas chromatography-mass spectrometry detection (GC-MS) to the separation and quantification of BPs in cosmetics products.

2. Results and Discussion

In the first stage of the studies, optimization of conditions of the chromatographic analysis (GC-MS) for the determination of BP-1, BP-3, and BP-8 (standard solution in methanol) was performed. The limits of detection of the analytes were determined, calibration curves were prepared, and precision of the chromatographic analysis was determined. All compounds showed good linearity ($R^2 > 0.984$) by direct injection with a linear range of 2.5–600 μg L^{-1}. The limits of detection (LODs), calculated as signal-to-noise ratio (S/N) of 3, ranged from 34 to 70 ng mL^{-1} for the MS(SCAN) detector and from 13 to 24 ng mL^{-1} for the MS(SIM) detector. The instrumental precision as relative standard deviations (RSD) was lower than 6.3% (at concentration of 100 μg L^{-1}).

Satisfactory parameters of the chromatographic analysis allowed us to conduct research on the extraction methods. In the case of water samples, three benzophenones were tested: BP-1, BP-3, and BP-8. However, in the case of cosmetics samples, only one of the benzophenones—BP-3—was tested. The reason for this was that during inspection of cosmetics in local stores it turned out that only cosmetics containing BP-3 were available. However, the results of research indicate that BP-1 and BP-8 will behave during extraction similarly to BP-3. The selectivity of the method was assessed by the absence of interfering chromatographic peaks at the retention time of the target analytes (Figure 1).

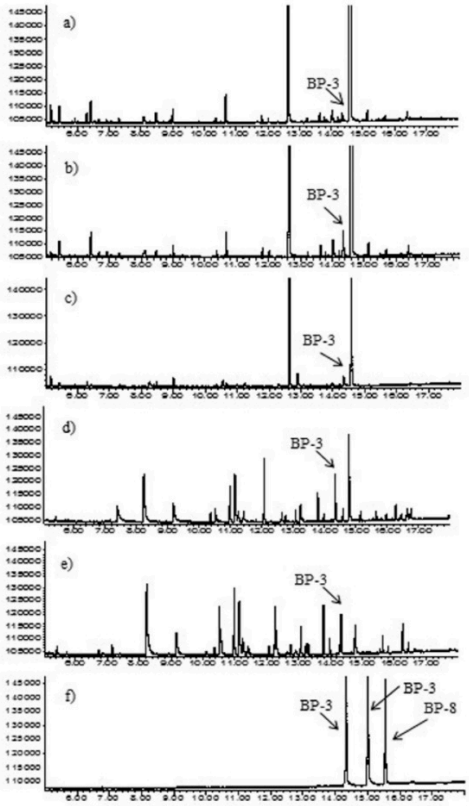


Figure 1. Chromatograms of (**a**) hair mask sample—SPE method; (**b**) hair mask sample with addition of BP-3 standard—SPE method; (**c**) hair mask sample—MEPS method; (**d**) shampoo sample–SPE method; (**e**) shampoo sample—MEPS method; (**f**) standard solution of BPs at concentration of 50 μg mL^{-1}.

2.1. Optimization of SPE Conditions

The determination of BPs in water samples using SPE (500 mg C18 cartridges) was performed according to the procedure presented by Giokas et al. [18], who obtained the recovery rate for BP-3 at the level of 95–97% for the natural water samples. Using this procedure, we obtained recovery rates for three BPs ranging from 101 to 107%. The accuracy, expressed as recovery percentage (%) of the SPE-GC-MS method, was calculated as the ratio of the found concentration to the expected concentration (concentration 5 μg L^{-1}) after spiking a sample. The repeatability, expressed as relative standard deviation (%RSD) of peak areas, was evaluated by applying the proposed method in six replicates at two concentration levels (5.0 and 50.0 μg L^{-1}) of standard solutions containing the target analytes. The intra- and inter-day precision values for all analytes in water samples were lower than 11.8 and 13.4%, respectively, highlighting the good reproducibility and repeatability of the method (Table 1). The accuracy and precision were satisfactory and therefore no modifications were introduced into the procedure.

To examine the enrichment factor (EF), the ratio of the final concentration of analytes in the solvent after extraction to the concentration of analytes in water solution subjected to the SPE process under optimum conditions was calculated. This value was also corrected by a degree of recovery. The use of large sample volumes (500 mL) results in a high enrichment factor (~1000), which has an impact on the possibility of determining BPs at low concentration levels, the values of which depend on the type of detection used. Parameters characterizing the SPE method are presented in Table 1. When using the MS detector, LODs were obtained at low concentration levels, ranging from 34 to 67 ng L^{-1}. According to the literature data, 10 times lower LODs values can be obtained when using the MS-MS detector [20]. LODs values obtained for the SPE technique are comparable to other methods, i.e., SPME (0.15–8.2 ng L^{-1}) [20,21], SBSE, and DLLME (2–11 ng L^{-1}) [26,28], where BPs were derivatised and analyzed by GC -MS/MS.

2.2. Optimization of MEPS Conditions

BIN with C18 filling was used to investigate the possibility of using the MEPS technique for the determination of BPs in samples of water and cosmetics. As the investigations on the use of the C18 deposit in the SPE technique showed very high recoveries of ~100%, we decided to base on the parameters of this procedure. Basing on the procedure used for the SPE, ethyl acetate (EA) and dichloromethane (DCM) were used as the conditioning solvents (250 μL) and 100 μL of the EA/DCM mixture (1:1, *v*/*v*) for elution in the MEPS method. Using these parameters, the recovery was only 70–80% for BP-1 and BP-8 and 90% for BP-3.

For this reason, it was checked whether the sorption bed was overloaded (1 and 2 mL of sample) and whether the amount of eluent was sufficient to elute the analytes (50 and 100 μL) with the use of different eluents (DCM, EA, and EA/DCM mixture (1:1, *v*/*v*)). An effect of these variable parameters on the peak areas is presented in Figure 2.

Table 1. Characteristics of the SPE-GC-MS and MEPS-GC-MS methods for the determination of BPs in water samples.

Analytes	SPE							MEPS						
	Intra-Day (RSD, %) (n = 6)		Inter-Day. (RSD, %) (n = 6)		LOD [b], (μg/L)	Recovery [a], (%)	EF [c]	Intra-Day (RSD, %) (n = 6)		Inter-Day (RSD, %) (n = 6)		LOD [b], (μg/L)	Recovery [a], (%)	EF [d]
	5.0 (μg L^{-1})	50.0 (μg L^{-1})	5.0 (μg L^{-1})	50.0 (μg L^{-1})				5.0 (μg L^{-1})	50.0 (μg L^{-1})	5.0 (μg L^{-1})	50.0 (μg L^{-1})			
BP-1	9.0	7.7	10.8	8.0	0.034	101	1010	14.2	7.6	18.8	11.2	1.8	96	20
BP-3	8.2	11.2	8.6	11.0	0.050	105	1050	11.8	4.0	14.8	6.6	2.9	90	18
BP-8	11.8	11.0	10.9	13.4	0.067	107	1070	15.6	6.6	17.2	9.6	3.2	106	21

[a] BPs at conc. of 5 μg L^{-1}; [b] The determination limit (LOD) defined as three times the signal-to-noise ratio (S/N = 3); [c] Water volume 500 mL, eluent volume 0.5 mL; [d] Water volume 2 mL, eluent volume 0.1 mL; EF—enrichment factor.

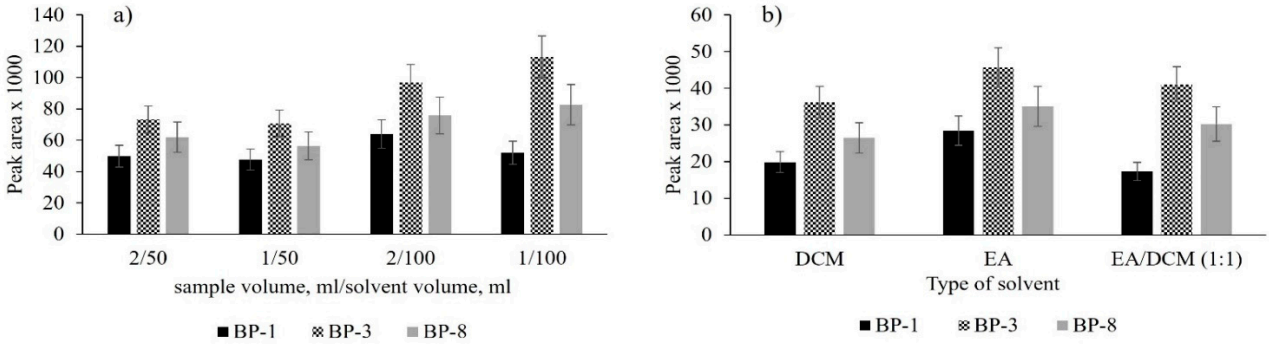


Figure 2. Optimization of MEPS method, sample spiked concentration 100 μg L^{-1}, (**a**) effect of sample volume (1, 2 mL) and eluent volume (50, 100 μL) (EA/DCM, 1:1, *v*/*v*); (**b**) selection of eluent (sample volume 1 mL, eluent volume 100 μL).

For the graphic presentation of the effect of the sample volume subjected to extraction and the extractant volume (Figure 2a), the results were converted to equal values of these volumes.

The presented results indicate that no overloading of the bed was found with the larger sample volume (2 mL) introduced. On the other hand, the volume of the eluent used for desorption has the greatest influence on the extraction efficiency.

The greater volume of solvent (100 μL) makes the elution step more efficient. In addition, it was observed in subsequent studies that elution of analytes with two portions of solvent (2 × 50 μL) increased its efficiency by ~12% compared to one-stage elution (1 × 100 μL).

For the study on an effect of solvent type on the extraction yield, 100 μL each of DCM, EA and an EA/DCM mixture (1:1, *v*/*v*) were used. The results of studies are presented in Figure 2b. Studies have shown that the type of solvent has a significant effect on the desorption stage. The best desorption effects are obtained when EA is used. Its efficiency of desorption is approximately 20–40% higher than that of the other solvents used, therefore it was used in further studies.

At predetermined, optimal extraction conditions, the degrees of recovery and the precision of the method, expressed as relative standard deviation (%RSD, n = 6) of peak areas, were determined. The results of the studies are presented in Table 1. Satisfactory recovery rates of 90, 96, and 106% were obtained for BP-3, BP-1, and BP-8, respectively. The intra- and inter-day precision of the MEPS method is high for low concentrations, ranging from 11.8 to 18.2%, while for higher concentrations it is satisfactory and ranges from 4.0 to 11.2%.

It was found that the MEPS technique has one significant disadvantage, i.e., a very low enrichment factor of about 20. It results from a very small amount of the analysed sample, the possible increase of which will not cause a large increase in the value of the enrichment factor. Therefore, the MEPS technique can only be used to determine higher concentrations of analytes in test samples. Table 1 shows the LODs of the tested BPs. These values confirm the earlier assumptions, as the LODs were 1.8–3.2 μg L^{-1}. Apart from the problem of the low enrichment factor, the MEPS technique has some very important advantages. These advantages are the small volume of solvent used and the small sample volume needed for the test. Additionally, MEPS is an easy, rapid (10 min), and not very labor-intensive process. The parameters of this method showing their advantages and disadvantages in comparison with other technics using GC-MS described in the literature are presented in Table 2.

Table 2. Comparison of proposed MEPS-GC-MS method to determine target analytes in water with other analytical methods reported in the literature.

Sample Preparation Technique	Matrix	LOD (ng L^{-1})	R (%)	RSD (%)	SAV [a] (mL)	SOV [b] (mL)	ET [c] (min)	EF	Reference
SPE-GC-MS/MS	water	0.3–1.0	67–73	1.8–3.0	100	6.1	-	700	[17]
SPE-GC-MS	water	3	95–97	5	500	20	60	50,000	[18]
MEPS-GC-MS	water	44.0–53.0	95–109	4–8	0.8	2	-	16	[32]
(DI)SPME-GC-MS/MS	water	0.15–3.0	80–115	6–13	10	-	30	-	[21]
(HS)SPME-GC-MS	water	9.0	-	<20%	40	-	125	-	[22]
(DI)SPME-GC-MS/MS	water	0.3–8.2	80–103	8.4–11	10	-	30	-	[20]
SBSE-LD-GC-MS	water, wastewater	2.0	28	1.3	100	0.2	510	140	[31]
SBSE-TD-GC-MS	water, wastewater	11.0	63	12–15	20	-	180	-	[28]
(DI)μ-SPE-GC-MS	water	0.5–2.0	85–96	4–9	10	-	10	-	[35]
SBSDμE-GC-MS	water	148	80–116	<12	25	-	50	-	[34]
FPSE-GC-MS/MS	water	4.5	88–110	9.2–12.0	30	20	3	-	[36]
MEPS-GC-MS	water	1.8–3.2	90–106	4.0–16	2	2	10	20	proposed method

[a] SAV—sample volume; [b] SOV—solvent capacity; [c] ET—extraction time.

When using SPE cartridges, the sorbent is discarded after use. In the MEPS method, the sorbent is used repeatedly. According to the manufacturer's information and literature reports, depending on the sample matrix, the MEPS-BIN can extract up to 100 samples with stable efficiency. We have conducted sorbent stability studies by comparing the effectiveness of the used BIN to the effectiveness of a new, unused (after conditioning) bed. In the case of analysis of BPs in water samples after ~100 extractions, the efficiency of the bed decreased by ~10%. However, in the case of analysis of the cosmetics solution after ~70 extractions, the extraction efficiency decreased by ~20%, followed by the BIN exchange.

2.3. Application of SPE and MEPS Methods for the Quantitative Determination of BP-3 in Cosmetics Samples

The developed SPE and MEPS methods, as described above, can be successfully applied to the determination of BPs in water samples. However, we decided to check whether they would also be suitable for the determination of BPs in cosmetics samples.

An analysis of the composition of cosmetics available in local stores and containing UV agents was performed. It was found that BP-3 was commonly found in cosmetics from the group of benzophenones. In the first stage of the studies, a hair mask containing BP-3 and a shampoo without UV filters were used. A hair mask is a cosmetic with a much higher density compared to a shampoo. The first stage of study on the application of the developed methods for the analysis of BP-3 in cosmetics consisted in the selection of the cosmetic:water ratio. The following cosmetic:water proportions were applied: (*m*/*v*)—1:10,000 for SPE and 1:1500 for MEPS. The preparation of cosmetics solutions in water consisted in weighing them and then dissolving them by mixing the solution with a magnetic stirrer. Dense samples of cosmetics (hair mask) required long mixing of the solution (30 min) to dissolve them completely, while dissolving the shampoo was much faster (15 min). The samples prepared by this method were subjected to SPE and MEPS extraction according to the procedures developed for water samples. In order to check the selectivity of the method and the possibility of BP-3 detection, the extracts were analyzed by chromatographic method.

The previously used parameters of the chromatographic analysis turned out to be suitable also for the analysis of cosmetics samples. Figure 1 shows exemplary chromatograms of extracts obtained after the preparation of cosmetics samples using the SPE and MEPS methods. The identification of BP-3 was confirmed by the internal standard method (Figure 1b) and by analysis performed with the MS detector.

Recovery and relative standard deviation (RSD) are the most important parameters of the tested methods (SPE and MEPS), allowing their use for the quantitative determination of BP-3 in cosmetics samples. These parameters were determined by testing a shampoo without UV filters. Two samples of the shampoo were prepared to which BP-3 was added in amounts of 0.033 and 0.330% and then the samples were prepared according to the procedures described above. The results of tests are presented in Table 3.

Table 3. Precision and accuracy of the SPE and MEPS methods obtained in determination of BP-3 in cosmetics samples.

Analytes	SPE						MEPS					
	Intra-Day (RSD, %) (n = 6)		Inter-Day (RSD, %) (n = 6)		Recovery (%)		Intra-Day (RSD, %) (n = 6)		Inter-Day (RSD, %) (n = 6)		Recovery (%)	
BP-3	11.5 [a]	12.4 [b]	13.8 [a]	14.0 [b]	69.5 [a]	58.2 [b]	3.9 [a]	14.4 [b]	6.6 [a]	15.5 [b]	69.7 [a]	44.0 [b]

[a] 0.033% (BP-3 in shampoo); [b] 0.330% (BP-3 in shampoo).

The results of both intra- and inter-day precision expressed as relative standard deviation (%RSD) for SPE and MEPS methods ranged from 3.9 to 15.5%. Considering the low concentration of BP-3 in the tested solution and the type of matrix tested, i.e., a cosmetic, these values can be considered satisfactory.

The accuracy of the methods (expressed as recovery, R) was calculated as the ratio of the found concentration to the expected concentration after spiking a sample. It was examined at the two concentration levels; every level was examined in three separate experiments. The recovery depends on the BP-3 content of the shampoo sample. In both cases, a higher recovery was obtained for lower concentrations (0.033%), amounting to ~70%. In contrast, the higher BP-3 content in the shampoo resulted in a significant reduction in recovery to 44 and 58% for MEPS and SPE, respectively. The recoveries for BP-3 from the shampoo sample were lower than in the case of the water samples, which proves the influence of the matrix on their values. It can be observed that the recovery is much lower for MEPS compared to SPE. This is probably due to a small amount of the sorption bed, and thus to the higher sensitivity to 'matrix effects'. The only solution is to prepare a water sample with a lower cosmetics content and to use a more sensitive detector, e.g., the MS-MS detector.

Due to the varied and 'rich' composition of cosmetics and relatively low levels of BP-3 recoveries (by SPE and MEPS methods), it was found that the most appropriate method for quantitative analysis of BPs in cosmetics would be the calibration method with standard addition (SA). The standard addition (SA) method is a powerful tool to minimize matrix effects and that enables precise and accurate determinations. It is also very important that the application of the SA method does not require the determination of recovery rate for each individual sample. However, it is laborious because it requires the preparation of a calibration curve for each sample. On the other hand, with respect to the MEPS method, in which the same sorbent is used many times, the phenomenon of the 'wear' of the bed will not have a major impact on the results.

A cosmetic (hair mask) containing information on the presence of BP-3 in its composition was used for the quantitative analysis. The quantitative analysis consisted in adding different amounts of BP-3 standard to the mask sample and analyzing these samples and the mask sample individually. The BP-3 content in the mask sample was calculated from the calculated value of the intersection of the calibration curve with the x axis.

The analyses were performed using two procedures (SPE and MEPS) for the same matrix in three repetitions. The linear correlation coefficients for the calibration curves

for both methods were $R^2 > 0.99$. Calibration curve equations and proportions of the prepared test samples were needed to calculate BP-3 content in the cosmetic (hair mask). The obtained mean results of studies were 0.059 and 0.065% for the SPE and MEPS methods, respectively. With the objective to demonstrate the equivalence in terms of precision and accuracy of the used methods, the Snedecor F-test and Student-t test were done. The results of the calculated parameters for both methods are presented in Table 4. No statistically significant differences were found between the precision and accuracy in the two methods.

Table 4. Statistical comparison between the two techniques by Snedecor *F*-test and Student-*t* test; determination of BP-3 content in hair mask sample.

Analyte	SPE Mean $\pm$ s_1 (%)	MEPS Mean $\pm$ s_2 (%)	*F* Ratio (F_{cr})	*t*-Values (t_{cr})
BP-3	0.059 $\pm$ 0.006	0.065 $\pm$ 0.004	2.25 (19.00)	1.440 (2.78)

$n_1 = n_2 = 3$; $v = 4$; For $\alpha = 0.05$ critical *F* value = 19.0 and critical *t* value = 2.776.

The suitability of both methods for the determination of BP-3 in cosmetics samples with different composition was also confirmed. The tests were performed on the following cosmetics: two different shampoo samples and two different samples of a hair mask containing BP-3 and a hair gel to which BP-3 was added in two concentrations. Analyses of BP-3 content in these samples were performed by SPE and MEPS methods using the calibration method described above. The results of quantitative analysis are presented in the form of a graph (Figure 3).

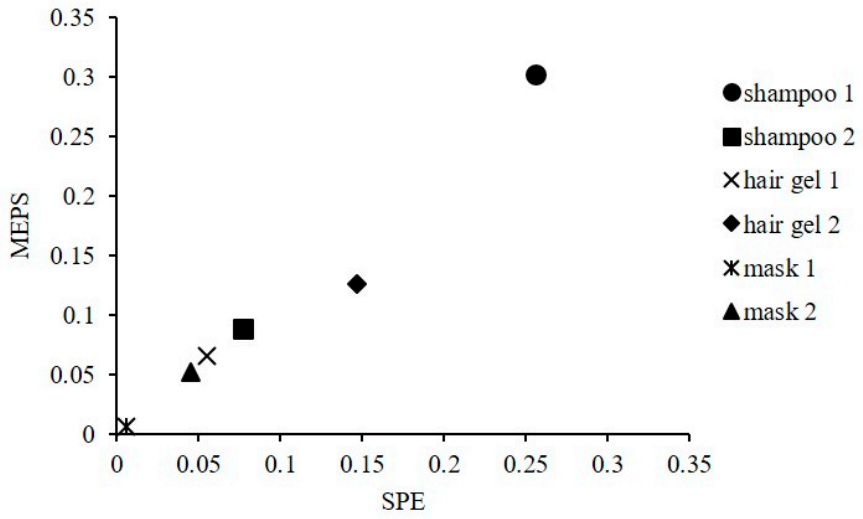


Figure 3. Comparison of BP-3 concentrations (%) in cosmetics samples determined by SPE and MEPS methods with GC-MS analysis.

A good correlation ($R^2 = 0.9676$) was demonstrated between the results obtained by the two methods (SPE and MEPS). The results confirmed that both applied analytical methods are suitable for the quantitative determination of BPs in cosmetics.

In Table 5, the characteristics of the SPE and MEPS methods with the application of GC-MS and other analytical methods with the application of GC/MS-MS reported in the literature for the determination BPs in cosmetics are presented.

Table 5. Comparison of proposed MEPS-GC-MS and SPE-GC-MS methods to determine target analytes in cosmetic samples with other analytical methods reported in the literature.

Sample Preparation Technique	LOD (%)	R (%)	RSD (%)	SAV [a] (mL)	SOV [b] (mL)	ET [c] (min)	Reference
GC-MS/MS	0.0018–0.27	101–105	0.69–1.13	0.1 g	0.7	40	[38]
PLE-GC-MS/MS	0.01–0.046	51.9–87.6	6.4–8.8	0.1 g	10	10	[37]
SPE-GC-MS	0.0003	58–70	12	0.1 g	15.5	60	proposed method
MEPS-GC-MS	0.001	44–70	14	0.3 g	2	15–30	proposed method

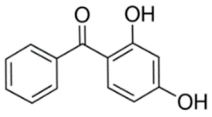
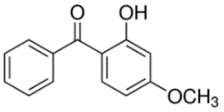
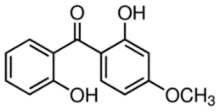
[a] SAV—sample volume; [b] SOV—solvent capacity; [c] ET—extraction time.

3. Materials and Methods

3.1. Materials and Reagents

BP-1, BP-3, and BP-8 were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Their structures and relevant physico-chemical properties are given in Table 6. HCl (32%), which was used for pH adjustment, was from Chempur (Piekary Śląskie, Poland). Ethyl acetate (EA), dichloromethane (DCM), and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). All compounds were analytical grade.

Table 6. Characteristics of the UV filters studied.

Analyte	Molecular Formula	CAS Number	Structure	Log K_{ow}	pKa
2,4-dihydoxybenophenone (BP-1)	$C_{13}H_{10}O_3$	131–56-6		2.96	7.1
2-hydroxy-4-methoxybenophenone (BP-3)	$C_{14}H_{12}O_3$	131-57-7		3.79	7.56
2,2′-dihydroxy-4-methoxybenzophenone (BP-8)	$C_{14}H_{12}O_4$	131-53-3		3.82	6.78

from: https://pubchem.ncbi.nlm.nih.gov (accessed on 30 October 2021).

The BP-3 was determined in different cosmetic products: mask for hair and shampoo with BP-3; shampoo and hair gel without BPs. The cosmetic products were purchased from local shops.

3.2. Standard Solutions

Stock standard solutions (each compound~1.0 mg L^{-1}) of BP-1, BP-3, and BP-8 were prepared in methanol and, additionally, a standard solution with BP-3 in methanol at a concentration of ~1.0 mg L^{-1} was used. These solutions were stored in the dark at 4 °C. From this standard solution, working solutions containing from 1.0 to 100.0 µg L^{-1} were prepared daily in water. The water solution was acidified (HCl) to pH 3.

The cosmetics products in amount of 0.1 g and 0.3 g (with accuracy to 0.0001 g) for SPE and MEPS methods, respectively, were spiked with standard solutions (BPs) of the appropriate concentrations and dissolved in 1000 mL water for the SPE method and in 500 mL water for the MEPS method. These solutions were mixed using the magnetic stirrer for 15–30 min and were prepared fresh every day.

3.3. SPE Procedure

The extraction of the analytes was performed using the C18 (1000 mg, 6 mL) cartridges obtained from Supelco (Bellefonte, PA, USA). The procedure was based as reported by Giokas et al. [30] and Lambropoulou et al. [17] with minor modifications.

The cartridge was conditioned with 5 mL EA and 5 mL DCM. Next, an aliquot of 500 mL of water or 100 mL of cosmetics solutions were pumped through the cartridge and air-dried under a vacuum. The analytes were eluted with 5 mL mixture of EA/DCM (1:1, *v*/*v*). The eluate was evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was redissolved in 0.5 mL EA and used in the GC analysis.

3.4. MEPS Procedure

Extraction was carried out by using a MEPS syringe (250 µL) packed with C18 (4 mg, mean particle size 45 µm, pore size 60 Å) sorbent from SGE (Trajan Scientific Australia Pty Ltd., Ringwood, Australia). Before being used for the first time, the packed sorbent was conditioned with 10 × 250 µL of EA, and then with 10 × 250 µL of DCM and 10 × 250 µL of EA/DCM (1:1, *v*/*v*).

The sorbent bed was conditioned by flushing 250 µL of EA/DCM (1:1, *v*/*v*) and 250 µL of ultrapure water before each extraction.

Next, 2000 µL of the sample was extracted by taking it from a vial and discarding to waste (eight cycles of 250 µL). Then, the sorbent was washed with ultrapure water (250 µL) and the cartridge was dried by pumping air through it (10 × 250 µL). The analytes were eluted with 100 µL of EA (2 × 50 µL). Finally, after elution the cartridge was washed three times with 250 µL of EA and three times with 250 µL of EA/DCM (1:1, *v*/*v*).

3.5. GC Analysis

Chromatographic analyses were performed using an Agilent 7890B (Agilent, Santa Clara, CA, USA), equipped with a split/splitless injector and multipurpose autosampler and an Agilent 5977B mass-selective detector.

The GC was fitted with a ZB-5-MS column (Zebron, Phenomenex Inc., Torrance, CA, USA), 30 m × 0.25 mm × 0.25 µm, containing (5% phenyl)-methylpolysiloxane.

The injector port was held at 270 °C and used in the splitless mode, and 2 µL injections were made. The temperature program used for the analysis was as follows: 100 °C, ramped at 10 °C/min to 260 °C and held for 4 min. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}.

Full-scan mass spectra were recorded with *m*/*z* range 50–300 in electron-impact mode at 70 eV.

The transfer line and ion source temperatures were set at 280 and 230 °C, respectively. The scan rate was 2.9 scan/s, cathode delay time 5 min. The SCAN mode was used for optimization studies and identification of analytes. Identification was accomplished using the NIST Mass Spectral Database (NIST MS Search 2.3) and by comparing retention times with standards. The select ion monitoring (SIM) mode was used only for the determination of the limits of detection.

4. Conclusions

The aim of this work was to develop easy, environmentally friendly, and rapid analytical methods for the determination of BPs in water samples and consumer cosmetics products. The methods are based on gas chromatography analysis and sorption of BPs on the C18 bed.

The studies have shown that both methods used, i.e., solid-phase extraction (SPE) and microextraction packing solid extraction (MEPS), are fully useful for the determination of benzophenones in water and cosmetics samples. The microextraction technique MEPS is an alternative to SPE in terms of benzophenones in water and cosmetics samples.

Both methods are characterized by essential advantages, i.e., in the case of SPE a significantly lower limit of detection for analytes were achieved, while MEPS is a fast

and simple method. Additionally, the use of organic solvents was drastically reduced. When determining BPs in cosmetics samples, it is very important to use the appropriate cosmetic:water proportions depending on the type of cosmetic and the expected BPs content in it. The applied calibration method with the standard addition is a guarantee of obtaining accurate results of quantitative analysis in cosmetics samples. Both of the methods are suitable for use in cosmetics quality control and environmental pollution assessment.

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Comparison of effectiveness and environmental impact of the selected methods for determination fatty acids in milk samples

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Abstract: Determination of the fatty acid profile in milk samples are one of the most important in food analysis. There are many methodologies for FA determination. The conventional procedure for determining the FA composition of milk is isolation of fat or indirect methylation, trans-methylation, extraction of fatty acids, and analysis by gas chromatography. In this study eight methods based on alkaline methylation were compared for the analysis fatty acids in cow's milk. The response factors (RF) for GC analysis using FID were calculated. For most acids RFs were close to 1, with the exception of short-chain fatty acids (C4:0-C8:0). To facilitate the selection of the method for the determination of fatty acids in milk samples, the methods were assessed using the environmental assessment tools of the analytical procedure: the Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and Analytical Greenness for Sample Preparation (AGREEprep). The method based on direct milk methylation received the highest scores. Omitting the lipid separation step has an impact on reducing the quantity of used toxic chemicals and reagents, and produces a smaller amount of waste, a much higher throughput, and a reduced cost analysis.

Keywords: environmental assessment tools, fatty acids, greenness, gas chromatography, milk fat

1. Introduction

Milk is a nutrient-rich food source in the human diet which contains lipids (dairy fat), high-quality protein, vitamins, minerals, and other bioactive components [1]. The most valuable component of milk is fat because it directly affects the nutritional value of the product, and also has an effect on sensory properties such as flavor and aroma [2]. Majority of milk lipids are in the form of triacylglycerols (TGA) which consist of a molecule of glycerol bound to three fatty acids (FA). When triacylglycerol is digested, then FAs become available for the human organism [3]. The FA composition is one of the most important indicators of the nutritional quality and physicochemical properties of milk fat. In addition to being related to human health, milk FAs can act as a potential indicator for the energy balance, metabolism and health of lactating cows, and can be used to predict new characteristics such as methane emissions and energy balance [4,5]. The FA composition of milk fat is influenced by various factors such as animal genotypes, diet, lactation stage and the physiological state of the cows. As a result, analysis of the composition of FA is of great importance in lipid-related research and for the dairy industry [6,7].

Generally, FAs are quantified according to their methyl esters (FAMEs) by gas chromatography-flame ionisation detector (GC-FID) or gas chromatography-mass spectrometry (GC-MS) following a sample preparation procedure, i.e., lipid extraction and transesterification. Methods used for methyl esterification include acid or base catalysis, as also acetyl chloride-methanol catalysis, BF3 or others agents. However, the acid- or alkaline-catalysis are most widely used for the determination of FA. The acid-catalysed methylation can convert FA from all lipid classes present in a sample into their correspondent

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FAME, but the methylation process is slower than alkaline-catalysed methylation, and could modify the profile of conjugated linoleic acids (CLA). In the case of alkaline-catalysed methylation, the main drawback this method is that only acyl moieties are converted to FAME. However, this does not cause important bias in the results because the proportion of lipids other than acyl moieties is low in milk fat. As mentioned above, the alkaline-catalysed methylation is faster than acid-catalysed methylation. Therefore, the alkaline-catalysed methylation is often recommended for milk FA profiling [6,8].

There are several methods of preparing samples of cow's milk for the determination of FA, e.g., the Folch method or Rose-Gottlieb method [9,10]. Methodological comparison studies for the determination of FAs are very influential, because milk samples require extreme care to get the lipid fraction, given that factors such as co-extraction of non-fatty component lipids and undesirable oxidation may influence the quality, and final quantification of the lipid fraction [11]. These methods are characterised by the consumption of toxic reagents, and are time-consuming and labor-intensive.

Therefore, it is important to evaluate the greenness of analytical procedures to assess, and if possible, reduce their impact on the environment and workers. Several tools are used in green analytical chemistry to address the environmental performance of an analytical procedure, including Analytical Eco-Scale, National Environmental Method Index (NEMI), Green Analytical Procedure Index (GAPI), and Analytical Greenness for Sample Preparation (AGREEprep), in which objective criteria related to analytical performance, sustainability, environmental impact and economic cost are evaluated through the definition of penalty points [12,13].

The main task of the presented study was to compare eight selected methods for the analysis of fat content in cow's milk. Additionally, the methods themselves were assessed using the environmental assessment tools of the analytical procedure. The results of these tests may be useful for researchers, and persons performing routine milk analyses in deciding on the choice of the analytical procedure.

2. Results and Discussion

2.1. Optimisation of GC-FID conditions

Preliminary investigations aimed for adjustment, and selection of the chromatographic conditions for the GC-FID analysis of FAs in milk. The research carried out by the manufacturer of the standard (37 FAMEs standard) and the column [14] were used to identify FAs. Separation and identification of over 20 different FAs was achieved, ranging from short-chain (C4:0) to long-chain (C22:0), particularly including various branched-chain FAs, C:18:1 isomers and CLAs. With the exception of compounds 17/18 (elaidic and oleic acids), all of the compounds were baseline separated. The unseparated peak from elaidic and oleic acids did not affect the purpose of this study. For the determination of the fatty acid profile in milk, fatty acids present in the sample above 0.01 g / 100 g FA were taken into account. A typical chromatogram of FAMEs from cow milk is shown in Figure 1.

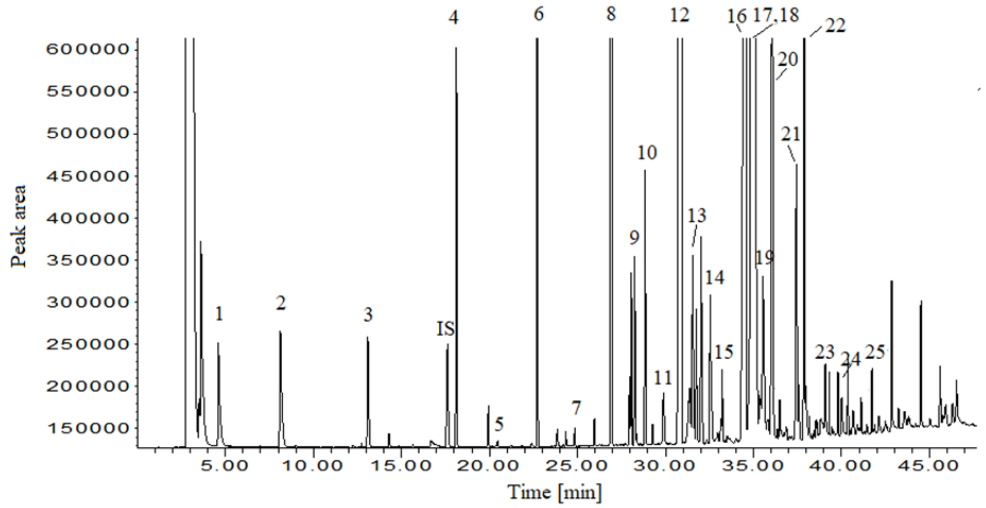


Figure 1. Typical chromatogram of milk lipid FAMEs. Peaks are: 1. butyric acid, 2. caproic acid, 3. caprylic acid, 4. capric acid, 5. undecanoic acid, 6. lauric acid, 7. tridecanoic acid, 8. myristic acid, 9. myristoleic acid, 10. pentadecanoic acid, 11. cis-100-pentadecanoic acid, 12. palmitic acid, 13. palmitoleic acid, 14. heptadecanoic acid, 15. cis-heptadecanoic acid, 16. stearic acid, 17. elaidic acid, 18. oleic acid, 19. linolelaidic acid, 20. linoleic acid, 21. arachidic acid, 22. linolenic acid, 23. cis-11-eicosenoic acid, 24. behenic acid, 25. arachidonic acid, IS. internal standard.

The used of FID for quantification of FAMEs is advantageous in relation to other detector types, because it is stable and easy to operate, possesses a wide dynamic range, and its introduction and maintenance costs are lower than for other types of detectors. The FID response is proportional to the number of carbon atoms that are burned. Heteroatoms (e.g. oxygen) in molecules usually reduce the FID signal which can make it worse accuracy in the quantification of fatty acids analysis. In these cases, in order to correct the responses of the detector is to use a response factor relative to each one of the analytes with respect to an internal standard [15,16].

In our research, experimental response factors (ERFs) for the quantification of individual FAs were determined by using standard FAMEs in the appropriate concentration. Table 1 gives the ERF and theoretical correction factors (TRFs) and the error factor (EF). The determined ERFs were compared with the TRFs. The ideal is to obtain results with an EF close to one, as in this way the results obtained will be highly accurate. Almost all of the ERFs were lower than the TRFs, which could have resulted from improper functioning of the GC system, purity of the standards, adsorption, decomposition, or discrimination of analytes during GC. In particular the difference is seen for C4:0 and C6:0, due to losses of volatility during the preparation of calibration solutions. With the exception of these two acids, the ERF results ranged from about 0.9-1.0. Therefore, we followed the recommendation of Bannon et al. [17] to use the theoretical factors in quantitative determinations of FAs, when there is a significant difference between REF and TRF.

The precision of the quantitative method was evaluated through the repeatability (intra-day) and reproducibility (inter-day) experiment. The intra-day of the method was established from six complete analyses of each sample under the same conditions in a day, and the inter-day was established from three complete analyses of each sample repeated on three consecutive days. Both intra-day and inter-day precision were satisfactory. The intra-day RSD ranged between 0.6 and 8.8% and inter-day RSD ranged between 0.5 and 10.0% (Table 1).

Table 1. Experimental and theoretical correction factors, error factor, intra-day and inter-day precision for the fatty acid in milk sample.

Fatty acid		ERF [1]	TRF [2]	EF [3]	Intra-day RSD (%)	Inter-day RSD (%)
butyric acid	C4:0	2.3260	1.5742	1.4776	7.5	8.0
caproic acid	C6:0	1.5308	1.3378	1.1443	6.6	2.4
caprylic acid	C8:0	1.1036	1.2195	0.9050	4.1	1.8
capric acid	C10:0	0.9640	1.2702	0.7589	2.5	1.7
undecanoic acid	C11:0	0.9501	1.1486	0.8272	2.3	2.9
lauric acid	C12:0	0.9433	1.1013	0.8566	0.6	1.3
oleic acid	C13:0	0.9549	1.0831	0.8817	1.2	0.9
myristic acid	C14:0	0.9678	1.0675	0.9066	1.6	0.8
myristoleic acid	C14:1	0.9835	1.0587	0.9290	1.6	1.0
pentadecylic acid	C15:0	0.9514	1.0540	0.9026	1.1	0.7
ginkgolic acid	C15:1	0.9439	1.0457	0.9027	1.0	1.7
palmitic acid	C16:0	0.9418	1.0422	0.9037	2.0	0.6
palmitoleic acid	C16:1	0.9909	1.0345	0.9579	1.7	0.5
heptadecanoic acid	C17:0	0.9418	1.0318	0.9127	1.9	1.1
10-heptadecenoic acid	C17:1	0.9019	1.0244	0.8804	1.7	4.5
stearic acid	C18:0	0.9183	1.0225	0.8981	3.5	1.6
elaidic acid + oleic acid	C18:1n9t + C18:1n9c	0.9000	1.0155	0.8863	1.8	1.1
linolealidic acid	C18:2n6c	0.9510	1.0087	0.9428	8.8	1.4
linoleic acid	C18:2n6t	0.9112	1.0087	0.9033	1.7	6.0
arachidic acid	C20:0	0.9813	1.0067	0.9748	1.4	6.6
alpha-linolenic acid	C18:3n3	0.9060	1.0017	0.9044	8.9	1.6
11-eicosenoic acid	C20:1n9	0.9359	1.0005	0.9354	7.3	10.0
behenic acid	C22:0	0.9326	0.9939	0.9384	2.9	2.7
arachidonic acid	C20:4n6	1.0020	0.9819	1.0205	2.4	5.8

[1] ERF = Experimental response factor, [2] TRF = Theoretical response factor, [3] EF = Error factor (ERF/TRF).

2.2. Comparison of preparation methods for FAMEs determination

In literature [4,6,18-20] and international standards [21-23], there are many of methods for the determination of FAs in a milk sample. Typically, FAMEs are quantified by GC following the multi-steps sample preparation procedure. The standard procedure for determining the profile of FAs in milk fat usually consists of the isolation of fat (extraction, centrifugation, evaporation), and the transesterification and extraction of FA.

Liu, Ezernieks, Rochfort, & Cocks [24] compared the transesterification methods of FA. This research shows the advantages and disadvantages of acid- and alkaline-catalysed transesterification. The results presented by the researchers show no significant differences ($p > 0.05$) between the different methods of methylation for the majority of FAs. However, due to the shorter time in alkaline-catalysed methylation, this type of transesterification was chosen in our research.

In our work, we used eight methods that differ mainly in the way of the fat isolation from milk sample. In order to obtain fat, extraction with organic solvents (methods A, G and G'), centrifugation (C, D), and centrifugation combined with evaporation (B) were used. In addition, we also used the method Additionally, we also used the method of directly methylation in milk (E and F), which was presented by Liu et al. [6]. The steps of sample preparation procedures used in our work was presented in Figure S1 (*Supplementary Material*).

As noted by Liu et al. [4], the use of crude fat isolated by centrifugation gives similar results in the relative proportion of each single FA compared to classical protocols requiring lipid extraction with organic solvents, which was also confirmed in our work (methods A, B, C and D). It turned out that these methods also did not differ significantly ($p < 0.05$) from methods A, B, C and D. For methods B, C and D, which using centrifugation of the milk sample to extract the milk fat, it was found that the milk fat contained a significant amount of water. In order to check the influence of the presence of water in the fat on the obtained results, the analysis of fresh fat (method B) and dried fat (method C) was performed. It was found that for most of the main FAMEs monitored there was no significant difference ($p > 0.05$) between fresh and dry fat. This suggests that although the centrifuged milk fat contains a significant amount of water, it does not affect the methylation reaction. Nevertheless, it should be emphasised that due to the presence of water and other components in the centrifuged fat layer, direct methylation of a weighed sample of crude fat does not allow for a reliable calculation of the absolute content of individual FAs in milk (mg FA/100g fat). In order to obtain the content of the FA in the milk fat, the content of water and other interfering substances would have to be precisely determined and included in the calculations.

The results of FA content in milk obtained by direct methylation methods (E and F) do not differ significantly ($p > 0.05$) from methods in which fat isolation (A-D) is used. Compared to method F, method E additionally used evaporation of the milk sample before the methylation step, which resulted in a slight loss of volatile FA. However, there did not affect significant ($p > 0.05$) for results. It should be pointed out that direct methylation of liquid milk (in E and F methods) did not make any additional interfering peaks as compared to the solvent-extracted 'clear' lipids. It is important because extra peaks can often co-elute, interfering with, and thus compromise the integration accuracy of peaks. Comparing the presented methods on the basis of the sample preparation steps (Figure S1, *Supplementary Material*), it can be concluded that the E and F methods are the safest, least time-consuming and cheapest methods. The skipping of the lipid extraction step and the possibility of direct transesterification of FAs is very advantageous compared to other methods.

Compared to the Folch (A) and modified PN-ISO 15885 (D) methods, which contained only lipids, the reaction matrices were much more complex when liquid milk was used directly for FAME preparation. However, despite the different behavior of the incubation samples (i.e., for method A – the reaction mixture was clear, in method B – milk solids clung to the wall of the vial, in method C – the reaction mixture was a cloudy suspension, for method E and F – residual milk solids could be seen in the reaction mixture), in all cases, upon adding hexane, phase separation was always achieved, and a transparent hexane extract obtained.

The overall GC-FID profile of FAMEs is similar for all major FA across the six above-mentioned compared methods. However, the results obtained for the G-G 'methods are significantly different ($p < 0.05$) from the other methods (A-F). This may be due to the multiple steps of the procedure, which may result in the loss of some FAs. Therefore, it can be concluded that incorrect results can be obtained when using method G for the determination of the FA profile.

Table 2. Fatty acid compositions of cow milk. Data are expressed as total mean (g/100 g FA) ± standard deviation of samples.

Nr of FA	FA	Method A	Method B	Method C	Method D	Method E	Method F	Method G	Method G′
1	C4:0	1.19±0.05^{c}	1.46±0.01^{a}	1.41±0.01^{a}	1.83±0.05^{d}	1.43±0.08^{a}	1.57±0.06^{a}	3.97±0.09^{b}	3.87±0.11^{b}
2	C6:0	1.69±0.09^{a}	1.79±0.14^{a}	1.68±0.00^{a}	2.24±0.04^{a}	1.83±0.12^{a}	1.90±0.02^{a}	2.05±0.10^{a}	2.15±0.80^{a}
3	C8:0	1.12±0.05^{a}	1.07±0.08^{ac}	1.02±0.04^{a}	1.27±0.03^{a}	1.19±0.07^{a}	1.11±0.02^{a}	0.97±0.07^{ab}	0.95±0.04^{bc}
4	C10:0	2.66±0.05^{a}	2.52±0.09^{a}	2.42±0.06^{ac}	2.72±0.02^{a}	2.79±0.05^{a}	2.84±0.09^{a}	2.06±0.15^{b}	2.16±0.19^{bc}
5	C11:0	0.06±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.07±0.00^{b}	0.08±0.00^{bc}	0.08±0.01^{c}	0.05±0.00^{d}	0.03±0.00^{e}
6	C12:0	3.31±0.02^{a}	3.13±0.02^{a}	3.06±0.06^{a}	3.19±0.01^{a}	3.38±0.15^{a}	3.35±0.15^{a}	2.53±0.16^{b}	2.46±0.11^{b}
7	C13:0	0.13±0.00^{a}	0.12±0.00^{a}	0.12±0.00^{a}	0.12±0.00^{a}	0.13±0.01^{a}	0.14±0.01^{a}	0.10±0.01^{b}	0.09±0.01^{b}
8	C14:0	11.99±0.04^{a}	11.57±0.14^{a}	11.46±0.18^{a}	11.26±0.06^{a}	11.93±0.40^{a}	11.50±0.37^{a}	9.77±0.38^{b}	9.54±0.39^{b}
9	C14:1	1.61±0.01^{a}	1.54±0.02^{a}	1.52±0.03^{a}	1.57±0.01^{a}	1.58±0.05^{a}	1.59±0.04^{a}	1.06±0.06^{b}	1.09±0.07^{b}
10	C15:0	1.30±0.00^{a}	1.27±0.02^{a}	1.27±0.02^{a}	1.25±0.01^{a}	1.30±0.04^{a}	1.25±0.02^{a}	1.13±0.03^{b}	1.11±0.02^{b}
11	C15:1	0.26±0.00^{a}	0.26±0.01^{a}	0.26±0.00^{a}	0.25±0.00^{a}	0.26±0.00^{a}	0.25±0.00^{a}	0.23±0.00^{b}	0.21±0.00^{b}
12	C16:0	31.34±0.10^{a}	32.11±0.79^{a}	31.87±0.83^{a}	30.49±0.15^{a}	31.58±0.48^{a}	31.71±0.30^{a}	29.73±0.18^{b}	29.52±0.27^{b}
13	C16:1	2.26±0.01^{a}	2.00.±0.04^{b}	2.01±0.04^{b}	2.01±0.03^{b}	2.08±0.16^{ab}	1.95±0.04^{b}	1.67±0.03^{c}	1.62±0.03^{c}
14	C17:0	0.69±0.01^{a}	0.71±0.02^{a}	0.72±0.01^{a}	0.63±0.00^{ac}	0.70±0.00^{a}	0.71±0.03^{a}	0.61±0.01^{bc}	0.58±0.01^{b}
15	C17:1	0.29±0.00^{ac}	0.27±0.01^{a}	0.28±0.02^{a}	0.29±0.00^{a}	0.28±0.00^{a}	0.27±0.01^{a}	0.33±0.00^{b}	0.31±0.00^{bc}
16	C18:0	10.53±0.09^{a}	11.3±0.39^{a}	11.27±0.38^{a}	10.23±0.00^{a}	10.17±0.10^{a}	10.50±0.15^{a}	13.61±0.67^{b}	13.29±0.35^{b}
17+18	C18:1n9t+C18:1n9c	25.76±0.31^{a}	25.7±0.63^{a}	26.35±0.42^{a}	26.31±0.09^{a}	25.35±0.44^{a}	25.69±0.72^{a}	24.69±0.85^{a}	24.89±0.47^{a}
19	C18:2n6c	2.25±0.05^{ac}	2.22±0.04^{ac}	2.24±0.01^{ac}	2.27±0.03^{ac}	2.19±0.02^{a}	2.21±0.01^{ab}	2.52±0.06^{d}	2.31±0.05^{bc}
20	C18:2n6t	0.49±0.01^{a}	0.49±0.01^{a}	0.51±0.01^{a}	0.49±0.00^{a}	0.48±0.01^{a}	0.47±0.03^{a}	1.05±0.08^{b}	1.03±0.04^{b}
21	C20:0	0.18±0.00^{a}	0.19±0.01^{a}	0.19±0.01^{ab}	0.16±0.00^{a}	0.18±0.00^{ab}	0.21±0.00^{bd}	0.26±0.01^{e}	0.23±0.01^{d}
22	C18:3n3	0.46±0.01^{a}	0.44±0.01^{a}	0.45±0.00^{a}	0.47±0.00^{a}	0.44±0.01^{a}	0.45±0.01^{a}	0.45±0.01^{a}	0.39±0.01^{b}
23	C20:1n9	0.53±0.01^{a}	0.54±0.01^{a}	0.54±0.01^{a}	0.55±0.01^{a}	0.52±0.01^{a}	0.54±0.01^{a}	0.76±0.05^{b}	0.72±0.06^{b}
24	C22:0	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.20±0.02^{b}	0.19±0.02^{b}
25	C20:4n6	0.18±0.00^{a}	0.18±0.00^{a}	0.18±0.00^{a}	0.19±0.01^{acd}	0.17±0.00^{a}	0.18±0.00^{ac}	0.21±0.01^{b}	0.20±0.01^{bd}
					Sums				

ΣSFA [1]	65.92[a]	66.35[a]	65.66[a]	65.60[a]	66.80[a]	66.90[a]	67.04[a]	67.11[a]
ΣUFA [2]	34.08[a]	33.65[a]	34.34[a]	34.40[a]	33.20[a]	33.10[a]	32.96[a]	32.89[a]
ΣMUFA [3]	30.70[a]	30.31[a]	30.97[a]	30.98[a]	30.08[ac]	30.29[ac]	28.73[bc]	28.89[bc]
ΣPUFA [4]	3.38[a]	3.33[a]	3.38[a]	3.42[a]	3.12[a]	3.13[a]	4.23[b]	3.99[b]

[1] ΣSFA = sum of saturated fatty acids; [2] ΣUFA = sum of unsaturated fatty acids; [3] ΣMUFA = sum of monounsaturated fatty acids; [4] ∑ PUFAs = sum of polyunsaturated fatty acids. Values are given as the means ± SD (n = 3). Different letters (a-e) in the same column indicate significant differences ($p < 0.05$).

2.3. Assessment of the method greenness

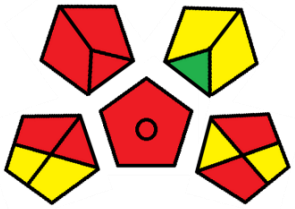
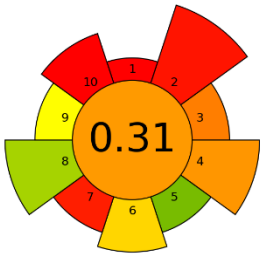
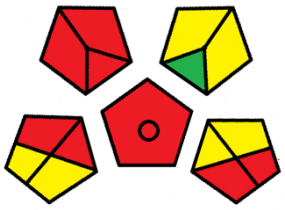
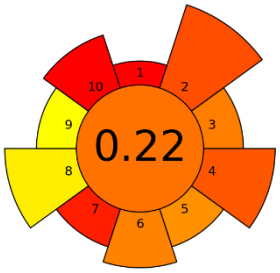
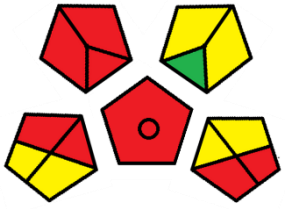
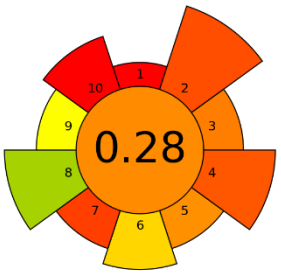
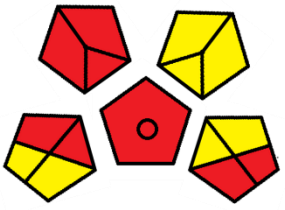
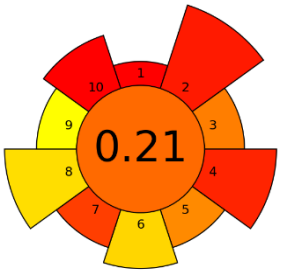
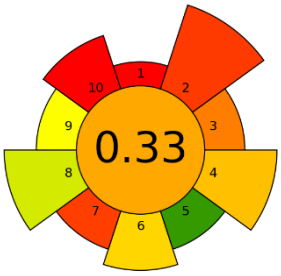
In order to facilitate the selection of the most advantageous method, it was decided to evaluate them in terms of their greenness. The objective is to evaluate the green impact of used methods sample preparation on operators, and the environment. This assessment includes the characteristics and amount of solvents and reagents used, amounts of waste produced, energy consumption, and the duration of the study.

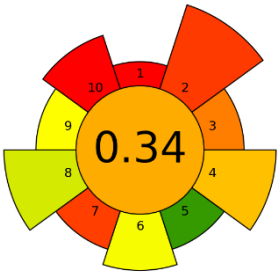
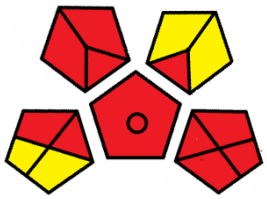
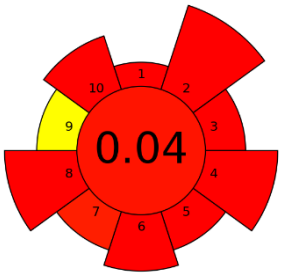
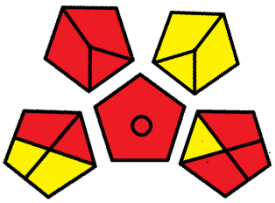
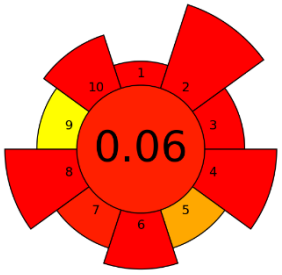
For the assessment we used three various matrices to estimate the greenness of our compared methods such as the Analytical Eco-scale, Green Analytical Procedure Index (GAPI) and Analytical Greenness Metric for Sample Preparation (AGREEprep). The Analytical Eco-Scale was introduced in 2012 by Gałuszka et al. [25]. In this method we calculate penalty points (PPs), which are assigned for high amounts, and high hazards connected with utilisation of chemicals, high energy consumption, occupational hazards and generation of wastes. The final result of an analytical Eco-Scale assessment is a number differing from 100 ('ideal green analysis') by a number of PPs. If the final score is above 75 points, it is considered 'excellent green analysis', but if it is between 50 and 75 points, it is considered 'acceptable green analysis'. The method with a final result below 50 points, is deemed 'inadequate green analytical procedure' [26,27]. The PPs for all the methods (A–G') are presented in Table 3, and the details of scoring are demonstrated in Table S1 (*Supplementary Material*).

The GAPI was introduced in 2018 by Płotka-Wasylka [28]. This is a new tool to assess the green character of the entire analytical procedure. GAPI's visual presentation allows for easy comparison of various methods and selecting from them the greenest. GAPI includes five pentagrams with 15 investigated parameters, that describe the environmental impact of every step of the analytical methodology such as sample collection and preparation, health and safety impact of reagents and compounds used, waste treatment, and energy consumption by instrumentation. The description of the pentagram is presented in Figure S2 (*Supplementary Material*). GAPI uses a three-level colour scale: green, yellow, and red to represent low, medium, and high ecological impact for each step. The greenest method is that possessing the highest number of green zones, and least number of red zones. The green assessment profiles for the methods using the GAPI tool are presented in Table 3. Detailed descriptions of GAPI parameters for the methods are shown in Table 2S (*Supplementary Material*).

Recently, in 2022, AGREE creators introduced a modification to it called AGREEprep [29]. The proposed metric tool gives prominence to sample preparation only. The AGREEprep was based on 10 categories (description in the *Supplementary Material*) of impact that were recalculated to 0–1 scale sub-scores. Assessment was also based on the possibility to differentiate between criteria importance by assigning them weights. The assessment produces a pictogram summarising the overall greenness of the method. The criteria of assessment evaluated, among others, the choice and use of solvents, materials and reagents, waste generation, energy consumption, sample size, and throughput [13]. The pictograms made in the AGREEprep assessment are presented in Table 3. Detailed reports of these assessments are available in *Supplementary Material*.

Table 3. The greenness profile of the employed (A – G') methods for analysis FAMEs in milk sample using Eco-Scale, GAPI and AGREEprep metrics.

Method	Analytical Eco-Scale score	GAPI pictogram	AGREEprep pictogram
A	70 acceptable green analysis	 (1 green, 6 yellow, 8 red)	
B	72 acceptable green analysis	 (1 green, 6 yellow, 8 red)	
C	72 acceptable green analysis	 (1 green, 6 yellow, 8 red)	
D	73 acceptable green analysis	 (0 green, 7 yellow, 8 red)	
E	71 acceptable green analysis	 (1 green, 7 yellow, 7 red)	

F	71 acceptable green analysis	 (1 green, 7 yellow, 7 red)	
G	24 inadequate green analysis	 (0 green, 4 yellow, 11 red)	
G′	45 inadequate green analysis	 (0 green, 6 yellow, 9 red)	

Taking into account the complexity of the matrix, which is the milk, and the number of analytes determined, as well as the necessity to perform 10erivatization, one cannot expect high grades of the greenness of these methods. According to Eco-Scale, the A-F methods achieved 70-73 points, which qualifies them as 'acceptable green analysis'. In contrast, the G and G′ methods obtained less than 50 PPs and therefore belong to the group of "inadequate green analysis".

Methods A, B and C were assessed identically by GAPI. On the other hand, slight differences in the evaluation of these methods are visible when using the Analytical Eco-Scale and AGREEprep methods. The E and F methods were rated the highest by all the evaluation methods used. In the case of these two methods, the highest convergence of results was obtained, which for Analytical Eco-Scale and GAPI are identical. Slight differences in their assessment can be noticed when using the AGREEprep, where the result for the E method was 0.33, and for the F method the result was 0.34. This slight difference is due to the fact that method F was created by simplifying method E by omitting the step of evaporating the water and derivatising the FA directly in the milk. From this it can be concluded that AGREEprep is the most accurate way to assess the 'greenness' of analytical methods. It is probably related to the greater influence of even a small amount of used reagents and other parameters on the calculated final result. However, in the case of Analytical Eco-Scale and GAPI, e.g., in the category of the amount of reagents used, the same score is obtained in the range of 10–100 ml. The E and F methods proposed by Liu et al. [6] are eco-friendly, mainly due to the use of a small amount of sample (200 µl) and small amounts of toxic reagents. Additionally and of great importance, they are the least labour-consuming and time-consuming.

The methods G and G′ [21] in the evaluation of greenness obtained very bad results. They obtained a large amount of PPs – 76, which resulted in the final result being 24. The modification performed by us (method G′) consisting in reducing the amount of the

sample used for the test and the amount of appropriate reagents by 90%, and lowering the PPs to 55, and the result was 45. However, both methods (G, G') have been classified by the Analytical Eco-Scale as 'inadequate green analytical procedure'. Their assessment by GAPI, and AGREEprep was also very disadvantageous. The 15-field GAPI pictograms contain 11 and 9 red fields for G and G' methods, respectively. Likewise, almost all AGREEprep pictograms are red and their ratings are very low at 0.04 (G) and 0.06 (G'). Such results are related to the use of large amounts of toxic solvents and waste, a large amount of sample for testing and the multistage and time-consuming of the procedure. It was not approved by any of the assessment methods used. This assessment allows for the conclusion that the use of this method should be avoided.

Summing up the evaluation of the greenness of the tested methods, it can be stated that it should be taken into account before making a decision on the selection of the sample preparation method for the routine analysis of FA content in milk samples. Generally, all the discussed tools can assess the 'greenness' of analytical protocols and have their inherent merits and drawbacks, and hence, the ideal solution is to implement two of them at least to extract the maximum possible information about analytical procedures.

3. Materials and Methods

3.1. Milk sample

Raw cow milk was purchased from a local farm (Pomerania, Poland). This sample was an aliquot from an afternoon milking of one cow; its total fat concentration was 3.9% as determined by infrared spectroscopy. Sample was frozen immediately after collection and stored at -18°C until use.

3.2. Chemicals and reagents

Solvents and chemicals used for lipid extraction and FAME preparation were of analytical grade. Chloroform, methanol, *n*-hexane, ethanol, *n*-pentane, ammonia, diethyl ether, anhydrous sodium sulphate, disodium hydrogen citrate sesquihydrate, potassium hydroxide and sodium hydroxide and the hexadecane (used as internal standards) were from Sigma Aldrich (Darmstadt, Germany). The standard mix of 37 FAMEs and standard mix of C4:0-C24:0 even carbon saturated FAMEs were purchased from Supelco (Bellefonte, PA, USA).

3.3. Lipid extraction and FAME preparation

The study compares eight methods of isolating fat from raw milk. All methods as prescribed in the literature and international standards.

Method A (Folch method) [4,9]: total lipids of raw milk (0.5 mL) were extracted twice by chloroform/methanol (2:1, v/v), and the organic phase was transferred to vial and evaporated to dryness under a stream of N_2. Next, 2.4 mL of derivatization reagent (0.2 M KOH/MeOH) was added, and sample was incubated at 50°C for 20 min. After cooling, 1 mL of water was added, and FAMEs formed were extracted into 1 mL of hexane, and subjected to GC-FID analysis.The total time of sample preparation was 60 min.

Method B [4]: raw milk (14 mL) was centrifuged for 20 min and 25-30 mg of crude fat was weighted into a vial, and dried under a stream of N_2 for 60 min before methylation. The methylation reaction and the rest of the method were performed as described in the Method A. The total time of sample preparation was 120 min.

Method C [4]: raw milk (14 mL) was centrifuged for 20 min and 25-30 mg of crude fat was weighted into a vial, and directly subjected to methylation. The methylation

reaction and the rest of the method were performed as described in the Method A. The total time of sample preparation was 60 min.

Method D [30]: raw milk (15 mL) was centrifuged for 20 min and 50 mg of crude fat. Then, 5 mL of 5% (m/v) CH_3ONa solution was added. Next, the tube was shaken well for 10 seconds. 180 seconds after the start time, tube was opened, and added 2 mL of hexane. 210 seconds after start time was added 10 mL of disodium hydrogen citrate and sodium chloride aqueous solution. Shaken gently for 30 s. Then, the sample was centrifuged for 20 min. Supernatant was subjected to GC-FID analysis. The total time of sample preparation was 60 min.

Method E [6]: fresh milk (200 μL) was measured into a vial and then dried in a heating block (40°C) under a stream of N_2 for approximately 15 min. The 2.5 mL of derivatization reagent (0.2M KOH/MeOH) was added, and a sample was incubated at 50°C for 30 min with occasional shaking. After cooling to room temperature, 1 mL of HCL (1 M) was added to each vial and FAMEs formed were extracted into 1 mL of hexane and analysed directly by GC-FID. The total time of sample preparation was 60 min.

Method F [6]: fresh milk (200 μL) was subjected directly to methylation in a 5 mL glass vial without any pre-treatment. The methylation reaction and the rest of the method were performed as described in the Method E. The total time of FAME preparation this method was 40 min.

Method G [31]: 100 mL of the milk sample was mixed with 80 mL of EtOH and 20 mL of NH_3 solution in a funnel. Then, 100 mL of diethyl ether was added, and the funnel was shaken vigorously for 1 min. The solution was stood to achieve phase separation.Next, the 100 mL of n-pentane was added to the solution in the funnel, and was mixed carefully. Then, after phase separation, the aqueous layer was discard. Next, a 100 mL of sodium sulfate solution was added to the organic phase. After phase separation, the aqueous layer was discard. The procedure with sodium sulfate solution repeated twice. Then, 10 g of anhydrous sodium sulfate was added to the organic phase, and the content was mixed carefully. The flask was stand for 10 min and its contents was filtered. Using the rotary evaporator and stream of N2, content of the flask was evaporated. Then, 100 mg of the sample was weighted and dissolved in a 5 mL of hexane and mixed. A 0.2 mL of the transesterification reagent (2 M KOH/MeOH) was added, and mixed with the vortex mixer for 1 min. After, the additional reaction time of 5 min, 0.5 g of solid sodium sulfate was added and mixed again. The test tube was centrifuged for 3 min at room temperature. Supernatant was subjected to the GC-FID analysis. The total time of sample preparation was 180 min.

Method G′ [31]: The preparation method was performed as described in Method G. However, in this method used 10 times smaller the amount of sample and reagents for preparation method. The total time of sample preparation was 180 min.

In order to illustrate the course of proceedings in the above-mentioned methods, a flow chart was created (Figure S1, *Supplementary Materials*).

3.4. GC analysis

Chromatographic analyses were performed using an Agilent 7890B (Agilent, Santa Clara, CA, USA), equipped with a flame ionization detector (FID), split/splitless injector, and multipurpose autosampler. The GC was fitted with a SP-2380 column, 30 m x 0.25 mm x 0.2 μm (Supelco, Bellefonte, PA, USA), with a constant flow of 1.0 mL/min helium as carrier gas.

The injector port was held at 230°C and used in the split mode using a split ratio of 10:1, and injection volumes were 1 μL. The detector temperature was 250°C. The oven temperature program was: 50°C, where it was held for 2 min, then increasing it at 4°C/min to 220°C and held for 15 min.

3.5. Statistical analysis

Peak areas were obtained by manual integral with Agilent ChemStation F.01.00.1903. The experiments were carried out at least three times, and the results were expressed as the mean ± standard deviation. The data were subjected to analysis of variance (ANOVA) and Tukey's test. Results were considered statistically significant at $p < 0.05$.

3.6. Calculation of fatty acid contents

FAMEs were identified by comparison of retention times with reference standards (37 FAMEs and C4:0-C24:0 even carbon saturated FAMEs, SUPELCO) analyzed under the same conditions.

Peak areas were corrected by correction factor (F_i) described in ISO 12966-4:2015 [30].

The correction factor, Fi, is then:

$$\mathbf{F_i} = \frac{\mathbf{m_i \ x \sum A}}{\mathbf{A_i \ x \sum m}}$$

where: m_i – is the mas of FAME, *i*, in the reference mixture,

$\sum A$ – is the sum of all areas of all FAMEs of the reference mixture,

A_i – is the area of FAME, *i*, in the reference mixture,

$\sum m$ – is the total of the masses of the various components, as FAMEs of the reference mixture.

For the sample, the mass fraction, w_i, in grams per 100 g of each FAME, *i*, is as given by formula:

$$\mathbf{w_i} = \frac{\mathbf{F_i \ x \ A_i}}{\mathbf{\sum (F_i \ x \ A_i)}}$$

The calculated value corresponds to the percentage of mass of the individual FA calculated as triacylglycerol per 100 g fat:

$$\mathbf{FA}(\%) = \frac{\mathbf{F_i \ x \ A_i}}{\mathbf{\sum (F_i \ x \ A_i)}} \mathbf{\ x \ 100}$$

5. Conclusions

The procedure of preparing a milk sample for the determination of the FA profile is usually multi-steps, which makes it labor-intensive and time-consuming. Most often it requires the separation of fat by extraction or centrifugation, the use of transesterification reactions and extraction of the separated acids. For this purpose, the use of solvents and toxic reagents, the consumption of energy, and the production of hazardous waste is necessary. There are many procedures available in the literature that differ mainly in the derivatising agent, such as alkaline or acidic agents, BF_3 and others. The paper presents the results of the comparison of studies on the FA profile in a sample of cow's milk determined with the use of eight methods available in the literature, that use alkaline methylation. In order to facilitate the selection of the method used for routine analyses of the FA profile in milk, an assessment of the environmental impact of these methods was made. For this purpose, three methods of greening assessment were used: Analytical Eco-scale, Green Analytical Procedure Index (GAPI) and Analytical Greenness Metric for Sample Preparation (AGREEprep). As expected, none of the methods belongs to the green procedures.

The method in which methylation of FA is carried out directly in milk was scored the highest. The environmental assessment tools of the analytical procedure should be effectively compared and incorporated as a standard in the development and validation of a new environmentally benign analytical method.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Scheme of the compared methods for the determination fatty acids in milk sample.; Figure S2: Green Analytical Procedure Index pictogram with description.; Table S1: Calculated PPs (Eco-Scale) for evaluated analytical procedures for FAMEs determination in milk samples.; Table S2: Green Analytical Procedure Index (GAPI) parameters for analytical procedures (A – D) for determination of FAME in milk samples.

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Supplementary Materials

Comparison of effectiveness and environmental impact of the selected methods for determination fatty acids in milk samples

Izabela Narloch and Grażyna Wejnerowska

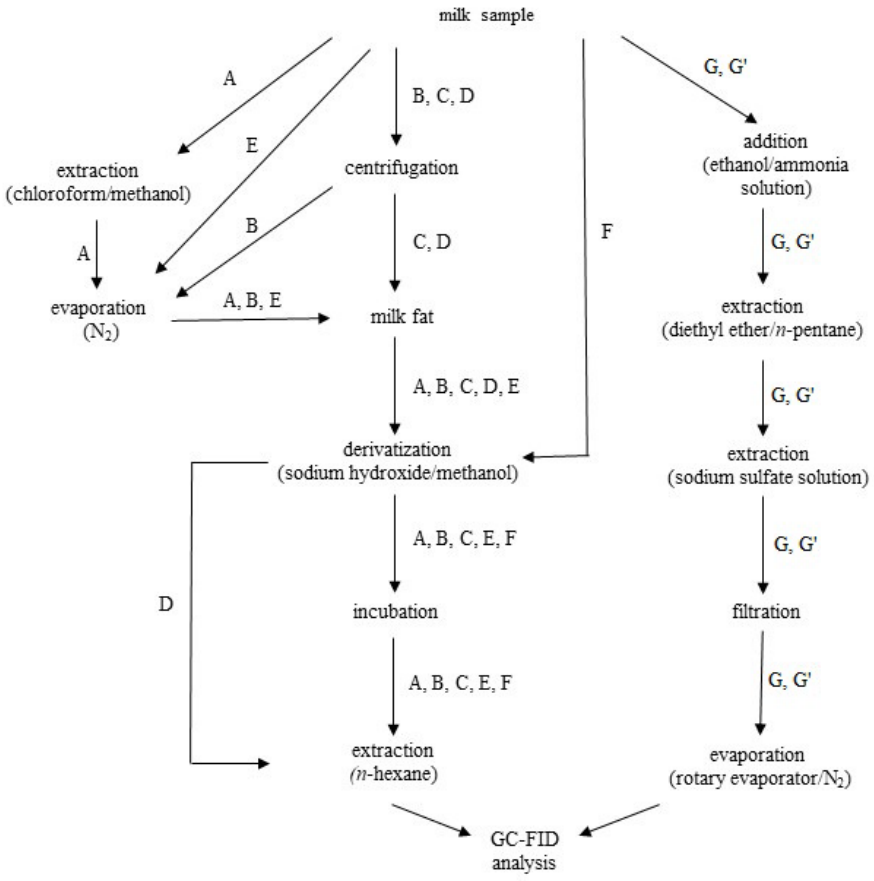


Figure S1. Scheme of the compared methods for the determination fatty acids in milk sample.

Table S1. Calculated PPs (Eco-Scale) for evaluated analytical procedures for FAMEs determination in milk samples (Procedures A - D).

Procedure A		**Procedure B**		**Procedure C**		**Procedure D**	
Reagents	**PPs**	**Reagents**	**PPs**	**Reagents**	**PPs**	**Reagents**	**PPs**
Methanol: 3.5 mL	6	Methanol: 2.1 mL	6	Methanol: 2.1 mL	6	Methanol: 4.75 mL	6
Chloroform: 3 mL	2	KOH: 0.3 g	2	KOH: 0.3 g	2	NaOH: 0.25 g	2
KOH: 0.3 g	2	n-hexane: 1 mL	8	n-hexane: 1 mL	8	n-hexane: 2 mL	8
n-hexane: 1 mL	8					Sodium hydrogencitrate/sodium chloride (3:2; *w:w*): 10 mL	0
	∑18		∑ 16		∑ 16		∑ 16
Instrument	**PPs**	**Instrument**	**PPs**	**Instrument**	**PPs**	**Instrument**	**PPs**
Transport	1	Transport	1	Transport	1	Transport	1
Sample storage (frozen)	0	Centrifugation (20 min; 5000 rpm)	1	Centrifugation (20 min; 5000 rpm)	1	Centrifugation (40 min; 5000 rpm)	1
Evaporation (N_2)	0	Evaporation (N_2, 60 min)	0	Incubation (50°C, 20 min)	1	GC-FID	1
Incubation (50°C, 20 min)	1	Incubation (50°C, 20 min)	1	GC-FID	1	Occupational hazard	0
Occupational hazard	3	Occupational hazard	0	Occupational hazard	0	Waste (>10 mL, no treatment)	8
GC-FID	1	GC-FID	1	Waste (>10 mL, no treatment)	8		
Waste (1-10 mL, no treatment)	6	Waste (>10 mL, no treatment)	8				
	∑12		∑ 12		∑12		∑ 11
Total PPs	**30**	**Total PPs**	**28**	**Total PPs**	**28**	**Total PPs**	**27**
Score – acceptable green analysis	**70**	**Score – acceptable green analysis**	**72**	**Score – acceptable green analysis**	**72**	**Score – acceptable green analysis**	**73**

Table S1. ***(cont.)*** Calculated PPs (Eco-scale) for evaluated analytical procedures for FAMEs determination in milk samples (Procedures E – G′).

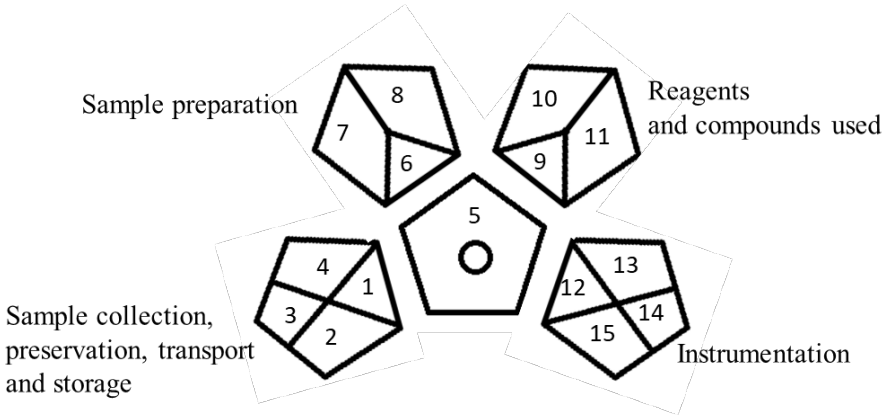
Procedure E		**Procedure F**		**Procedure G**		**Procedure G′**	
Reagents	**PPs**	**Reagents**	**PPs**	**Reagents**	**PPs**		
Methanol: 2.1 mL	6	Methanol: 2.1 mL	6	Ethanol: 80 mL	3	Ethanol: 8 mL	2
KOH: 0.3 g	2	KOH: 0.3 g	2	diethyl ether: 100 mL	12	diethyl ether: 10 mL	8
HCl: 1 mL	4	HCl: 1 mL	4	n-pentane: 100 mL	24	n-pentane: 10mL	16
n-hexane: 1 mL	8	n-hexane: 1 mL	8	NH_3 (aq.): 20 mL	12	NH_3 (aq.): 2 mL	6
				10% Na_2SO_4(aq.): 200 mL	0	10% Na_2SO_4(aq.): 20 mL	0
				n-hexane: 50 mL	4	n-hexane: 5 mL	2
				KOH: 0.16 g	2	KOH: 0.016 g	2
				Methanol: 2 mL	6	Methanol: 0.2 mL	6
	∑20		∑20		∑63		∑42
Instrument	**PPs**	**Instrument**	**PPs**	**Instrument**	**PPs**	**Instrument**	**PPs**
Transport	1	Transport	1	Transport	1	Transport	1
Incubation (50°C, 30 min)	1	Incubation (50°C, 30 min)	1	Rotary evaporator	0	Rotary evaporator	0
Evaporation (N_2, 40°C, 60 min)	0	Occupational hazard	0	Occupational hazard	3	Occupational hazard	3
Occupational hazard	0	GC-FID	1	GC-FID	1	GC-FID	1
GC-FID	1	Waste (1-10 mL, no treatment)	6	Waste (>10 mL, no treatment)	8	Waste (>10 mL, no treatment)	8
Waste (1-10 mL, no treatment)	6						
	∑9		∑9		∑13		∑13
Total PPs	**29**	**Total PPs**	**29**	**Total PPs**	**76**	**Total PPs**	**55**
Score – acceptable green analysis	**71**	**Score – acceptable green analysis**	**71**	**Score – inadequate green analysis**	**24**	**Score – inadequate green analysis**	**45**

Table S2. Green Analytical Procedure Index (GAPI) parameters for analytical procedures (A – D) for determination of FAME in milk samples.

Category	Method			
	A	**B**	**C**	**D**
Sample preparation				
Collection (1)	Off-line	Off-line	Off-line	Off-line
Preservation (2)	Physical (low temperature)	Physical (low temperature)	Physical (low temperature)	Physical (low temperature)
Transport (3)	Required	Required	Required	Required
Storage (4)	Samples must be frozen	Samples must be frozen	Samples must be frozen	Samples must be frozen
Type of method: direct or indirect (5)	Extraction required (liquid-liquid extraction)	Extraction required (liquid-liquid extraction)	Extraction required (liquid-liquid extraction)	Extraction required (liquid-liquid extraction)
Scale of extraction (6)	Macro-extraction	Macro-extraction	Macro-extraction	Macro-extraction
Solvents/reagents used (7)	Non green solvents/reagents used	Non green solvents/reagents used	Non green solvents/reagents used	Non green solvents/reagents used
Additional treatments (8)	Derivatization	Derivatization	Derivatization	Derivatization
Reagent and solvent				
Amount (9)	< 10 mL	<10 mL	<10 mL	10-100 ml
Health hazard (10)	NFPA = 2 or 3 (depends on the reagent)	NFPA = 2 or 3 (depends on the reagent)	NFPA = 2 or 3 (depends on the reagent)	NFPA = 2 or 3 (depends on the reagent)
Safety hazard (11)	Highest NFPA flammability or instability score is 2 or 3	Highest NFPA flammability or instability score is 2 or 3	Highest NFPA flammability or instability score is 2 or 3	Highest NFPA flammability or instability score is 2 or 3
Instrumentation				
Energy (12)	≤1.5 kWh per sample	≤1.5 kWh per sample	≤1.5 kWh per sample	≤1.5 kWh per sample
Occupational hazard (13)	Emission of vapours to the atmosphere	-	-	-
Waste (14)	1-10 mL	>10 mL	>10 mL	>10 mL
Waste treatment (15)	No treatment	No treatment	No treatment	No treatment
QUANTIFICATION	Yes	Yes	Yes	Yes

Table S2. (*cont.*) Green Analytical Procedure Index (GAPI) parameters for analytical procedures (E – G′) for determination of FAME in milk samples.

Category	Method			
	E	**F**	**G**	**G′**
Sample preparation				
Collection (1)	Off-line	Off-line	Off-line	Off-line
Preservation (2)	Physical (low temperature)	Physical (low temperature)	Physical (low temperature)	Physical (low temperature)
Transport (3)	Required	Required	Required	Required
Storage (4)	Samples must be frozen	Samples must be frozen	Samples must be frozen	Samples must be frozen
Type of method: direct or indirect (5)	Extraction required (liquid-liquid extraction)	Extraction required (liquid-liquid extraction)	Extraction required (liquid-liquid extraction)	Extraction required (liquid-liquid extraction)
Scale of extraction (6)	Macro-extraction	Macro-extraction	Macro-extraction	Macro-extraction
Solvents/reagents used (7)	Non green solvents/reagents used	Non green solvents/reagents used	Non green solvents/reagents used	Non green solvents/reagents used
Additional treatments (8)	Derivatization	Derivatization	Derivatization	Derivatization
Reagent and solvent				
Amount (9)	<10 mL	<10 mL	>100 mL	10-100 ml
Health hazard (10)	NFPA = 2 or 3 (depends on the reagent)	NFPA = 2 or 3 (depends on the reagent)	NFPA = 2 or 3 (depends on the reagent)	NFPA = 2 or 3 (depends on the reagent)
Safety hazard (11)	Highest NFPA flammability or instability score is 2 or 3	Highest NFPA flammability or instability score is 2 or 3	Highest NFPA flammability or instability score is 2 or 3	Highest NFPA flammability or instability score is 2 or 3
Instrumentation				
Energy (12)	≤1.5 kWh per sample	≤1.5 kWh per sample	>1.5 kWh per sample	≤1.5 kWh per sample
Occupational hazard (13)	-	-	Emission of vapours to the atmosphere	Emission of vapours to the atmosphere
Waste (14)	1-10 mL	1-10 mL	>10 mL	>10 mL
Waste treatment (15)	No treatment	No treatment	No treatment	No treatment
QUANTIFICATION	Yes	Yes	Yes	Yes



Sample preparation
1 Collection
2 Preservation
3 Transport
4 Storage
5 Type of method: direct or indirect
6 Scale of extraction
7 Solvents/reagents used
8 Additional treatments

Reagent and solvents
9 Amount
10 Health hazard
11 Safety hazard

Instrumentation
12 Energy
13 Occupational hazard
14 Waste
15 Waste treatment

Figure S2. Green Analytical Procedure Index pictogram with description.

Criteria and scores' calculation for AGREEprep [25]

The assessment criteria are based on the ten principles of green sample preparation given as below:

1. Favor in situ sample preparation
2. Use safer solvents and reagents
3. Target sustainable, reusable, and renewable materials
4. Minimize waste
5. Minimize sample, chemical and material amounts
6. Maximize sample throughput
7. Integrate steps and promote automation
8. Minimize energy consumption
9. Choose the greenest possible post-sample preparation
configuration for analysis
10. Ensure safe procedures for the operator

Reference:

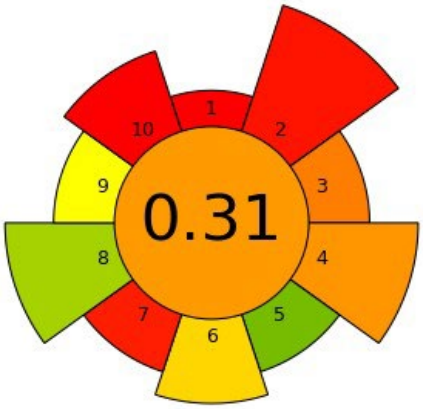
[25] Wojnowski, W.; Tobiszewska, M.; Pena-Pereira, F.; Psillakis, E. AGREEprep–Analytical greenness metric for sample preparation. *Trends in Anal. Chem.* **2022**, *149*, 11655.

AGREEprep

Analytical Greenness Metric
for Sample Preparation

METHOD A

16/07/2022 17:58:27



#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 7.4	0.04	5
3.	**Sustainability and renewability of materials** 25-50% of reagents and materials are sustainable or renewable	0.25	2
4.	**Waste** Mass [g] or volume [mL] of waste: 7.9	0.3	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 0.5	0.77	2
6.	**Sample throughput** Hourly sample throughput: 6	0.42	3
7.	**Integration and automation** No. of sample prep. steps: 5 steps; degree if automation: Manual systems	0.06	2
8.	**Energy consumption**	0.68	4

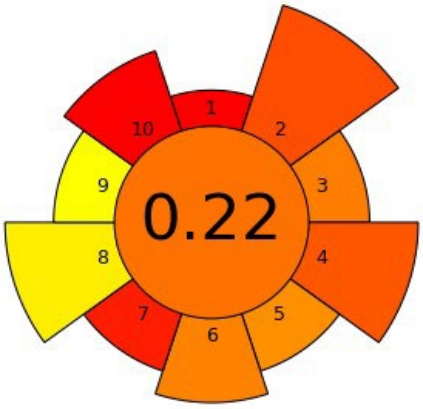
	Approximate energy consumption per analysis [W]: 35		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation



METHOD B

16/07/2022 18:22:01

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 3.4	0.16	5
3.	**Sustainability and renewability of materials** 25-50% of reagents and materials are sustainable or renewable	0.25	2
4.	**Waste** Mass [g] or volume [mL] of waste: 17.4	0.17	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 14	0.28	2
6.	**Sample throughput** Hourly sample throughput: 3	0.26	3
7.	**Integration and automation** No. of sample prep. steps: 5 steps; degree if automation: Manual systems	0.06	2
8.	**Energy consumption**	0.47	4

	Approximate energy consumption per analysis [W]: 80		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation

METHOD C

16/07/2022 18:26:30

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 3.4	0.16	5
3.	**Sustainability and renewability of materials** 25-50% of reagents and materials are sustainable or renewable	0.25	2
4.	**Waste** Mass [g] or volume [mL] of waste: 17.4	0.17	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 14	0.28	2
6.	**Sample throughput** Hourly sample throughput: 6	0.42	3
7.	**Integration and automation** No. of sample prep. steps: 4 steps; degree if automation: Manual systems	0.12	2
8.	**Energy consumption**	0.68	4

	Approximate energy consumption per analysis [W]: 35		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation

METHOD D

16/07/2022 18:26:30

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 3.4	0.16	5
3.	**Sustainability and renewability of materials** 25-50% of reagents and materials are sustainable or renewable	0.25	2
4.	**Waste** Mass [g] or volume [mL] of waste: 17.4	0.17	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 14	0.28	2
6.	**Sample throughput** Hourly sample throughput: 6	0.42	3
7.	**Integration and automation** No. of sample prep. steps: 4 steps; degree if automation: Manual systems	0.12	2
8.	**Energy consumption**	0.68	4

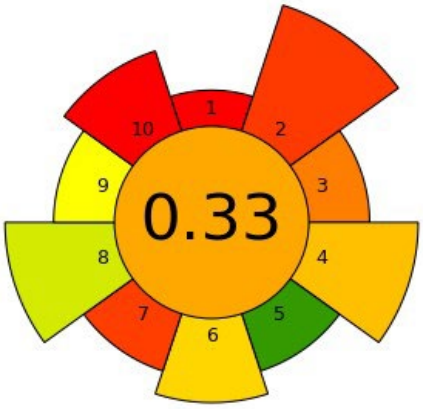
	Approximate energy consumption per analysis [W]: 35		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation



METHOD E

16/07/2022 18:47:36

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 4.5	0.12	5
3.	**Sustainability and renewability of materials** 25-50% of reagents and materials are sustainable or renewable	0.25	2
4.	**Waste** Mass [g] or volume [mL] of waste: 4.7	0.38	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 0.2	0.9	2
6.	**Sample throughput** Hourly sample throughput: 6	0.42	3
7.	**Integration and automation** No. of sample prep. steps: 4 steps; degree if automation: Manual systems	0.12	2
8.	**Energy consumption**	0.59	4

	Approximate energy consumption per analysis [W]: 50		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

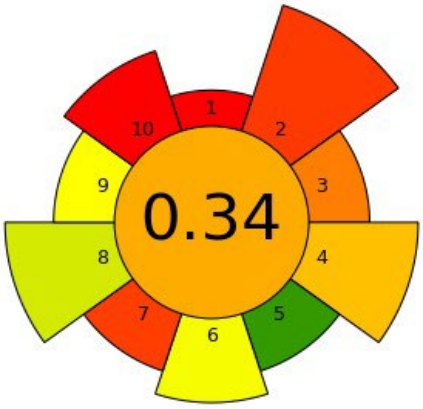
10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation

METHOD F

16/07/2022 18:54:36



#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 4.5	0.12	5
3.	**Sustainability and renewability of materials** 25-50% of reagents and materials are sustainable or renewable	0.25	2
4.	**Waste** Mass [g] or volume [mL] of waste: 4.7	0.38	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 0.2	0.9	2
6.	**Sample throughput** Hourly sample throughput: 9	0.52	3
7.	**Integration and automation** No. of sample prep. steps: 4 steps; degree if automation: Manual systems	0.12	2
8.	**Energy consumption**	0.59	4

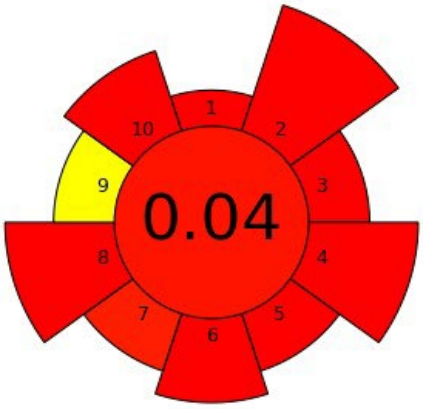
	Approximate energy consumption per analysis [W]: 50		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation



METHOD G

20/07/2022 11:13:08

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 352	0.0	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 750	0.0	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 100	0.0	2
6.	**Sample throughput** Hourly sample throughput: 0.7	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 5 steps; degree if automation: Manual systems	0.06	2
8.	**Energy consumption**	0.0	4

	Approximate energy consumption per analysis [W]: 1640		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

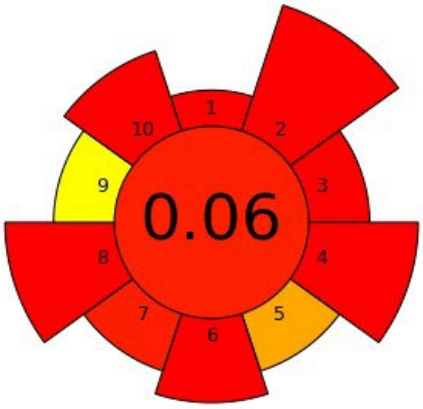
10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

AGREEprep

Analytical Greenness Metric
for Sample Preparation

METHOD G'

20/07/2022 11:16:41



#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 35	0.0	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 75	0.0	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 10	0.33	2
6.	**Sample throughput** Hourly sample throughput: 1	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 5 steps; degree if automation: Manual systems	0.06	2
8.	**Energy consumption**	0.0	4

	Approximate energy consumption per analysis [W]: 1000		

9.	**Post-sample preparation configuration for analysis**	0.5	2
	GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.		

10.	**Operator's safety**	0.0	3
	No. of distinct hazards: 4 or more hazards		

Publikacja naukowa [P4]

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Article

A Comparative Analysis on the Environmental Impact of Selected Methods for Determining the Profile of Fatty Acids in Cheese

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Abstract: The fatty acid profile of cheese influences its sensory parameters, such as color, texture, or flavor. Examining the fatty acid profile also helps to assess the nutritional value of the cheese that is being tested. However, the determination of fatty acids in cheese samples is a multi-stage and time-consuming task. In addition, large amounts of toxic organic solvents are used to prepare samples for analysis purposes. This paper presents the results of a study to determine the fatty acid profile of yellow cheese samples. Six different methods of sample preparation were compared for analysis purposes. The profile of fatty acids was determined using gas chromatography with flame ionization detection (GC-FID). The study showed significant differences ($p > 0.05$) in the resulting fatty acid profile between the methods used. It was found that the most reliable fatty acid profile results were obtained using methods derived from the Folch method. In addition, tools such as the Analytical Eco-Scale tool and the Analytical Greenness Metric for Sample Preparation (AGREEprep) tool were used to assess the greenness of the methods used. In the case of the Analytical Eco-Scale tool, all six methods scored 'acceptable green analysis' with scores ranging from 61 to 73. However, an evaluation of methods using the AGREEprep metric showed that the results of the methods (0.13–0.27) did not show the "greenness" of the analytical methods.

Keywords: dairy products; environmental assessment tools; food analysis; food composition; gas chromatography; greenness

Citation: Narloch, I.; Wejnerowska, G. A Comparative Analysis on the Environmental Impact of Selected Methods for Determining the Profile of Fatty Acids in Cheese. *Molecules* **2023**, *28*, 4981. https://doi.org/10.3390/molecules28134981

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1. Introduction

Cheese is a nutrient-rich food source of health-promoting compounds in the human diet. Fatty acid (FA) analysis of cheese is important in determining its nutritional value (labelling). In addition, FA analysis helps to examine cheese technology and is used to study the influence of various factors on the FA profile in milk (diet and breed of animals, season, etc.) [1,2]. Cheese's nutritional and sensory values are influenced by many factors, including milk characteristics, starter cultures, and technological processing [3,4]. Cheese characteristics shown are affected by the FA profile of milk used in its production. The degree of unsaturation of FA influences the texture of cheese. The higher the unsaturation, the softer the texture of the cheese. In the case of flavor, rancidity increases when the free FAs are released. Additionally, a pungent flavor is associated with a higher amount of short-chain FAs [5–8].

The complexity of the cheese matrix due to FA profile analysis requires the use of appropriate analytical procedures. The selection of appropriate methods plays a critical role in the efficient (qualitative and quantitative) extraction of major and minor lipids. Typically, these methods involve the use of large amounts of toxic solvents and therefore generate a lot of waste, and are laborious and time-consuming. Several evaluation methods allow the selection of the most efficient method for FA analysis in the cheese sample in terms of the greenness of analytical procedures. Several tools are employed in green analytical

chemistry to study the environmental performance of an analytical procedure, including the Analytical Eco-Scale tool, the National Environmental Methods Index (NEMI), the Green Analytical Procedure Index (GAPI), the Analytical Greenness (AGREE) tool, and the newer Analytical Greenness Metric for Sample Preparation (AGREEprep) tool. Objective criteria related to analytical performance, environmental impact, sustainability, and economic cost are evaluated by these tools through the definition of penalty points [9–16]. This makes it possible to choose the most environmentally friendly analytical procedure, which is very important nowadays for both ecological and also economic and analytical reasons.

In the literature, there are several methods available for preparing cheese samples for FA profile studies, but none compare their impact on the test results. In order to confirm the reliability of the authors' research, six methods were selected from those available in the literature to compare their results. In addition, these methods were evaluated using environmental assessment tools, which helped to evaluate the environmental impact of these procedures. The conclusions from this study are intended to draw the attention of researchers to analytical problems and ecological aspects when performing cheese analysis.

2. Results and Discussion

2.1. Optimization of GC-FID Conditions

The chromatographic conditions were previously optimized and validated for the GC-FID analysis of FA in cheese. Peaks were identified using retention times obtained from research carried out by the manufacturer on the standard mixture purchased from Supelco (37-component FAME mixture) with the column [17]. The FAMEs were separated according to the carbon number (the number of carbon atoms in the FA chain, excluding the methyl ester carbon) and the degree of unsaturation. Additionally, the position of the double bond(s) and the geometric configuration (cis/trans) are also important parameters. Their determination adds extra information to the characterization of the lipid fraction in cheese. In our study, 23 different FAs were separated and identified, ranging from short-chain (C4:0) to long-chain (C20:1n9) FAs. A good separation was obtained, except for the following compounds: cis- and trans-C18:1 (elaidic and oleic acids), which were coeluted. A typical chromatogram of FAMEs from cow cheese is shown in Figure 1.

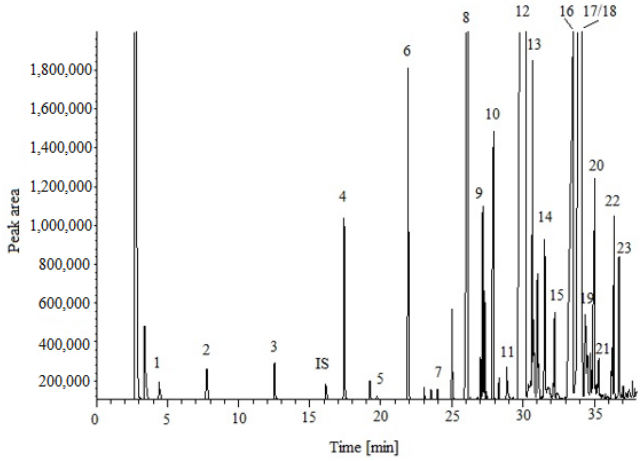


Figure 1. Exemplary chromatogram of cheese lipid FAMEs (the sample was prepared in accordance with method C). The peaks are: 1. butyric acid, 2. caproic acid, 3. caprylic acid, 4. capric acid, 5. undecanoic acid, 6. lauric acid, 7. tridecanoic acid, 8. myristic acid, 9. myristoleic acid, 10. pentadecanoic acid, 11. cis-100-pentadecanoic acid, 12. palmitic acid, 13. palmitoleic acid, 14. heptadecanoic acid, 15. cis-heptadecanoic acid, 16. stearic acid, 17. elaidic acid, 18. oleic acid, 19. linolealidic acid, 20. linoleic acid, 21. arachidic acid, 22. linolenic acid, and 23. cis-11-eicosenoic acid. IS—internal standard.

GC-FID is one of the most robust techniques used to detect various FAs. MS-based detection (e.g., GC-MS) is a more sensitive alternative to FID, but FID is advantageous

due to its stability, wide dynamic range, and introduction and maintenance costs. The FID signal is proportional to the number of carbon atoms in a hydrocarbon molecule. In the case of determining the FA percentage (in the wide FA range from C4:0 to C20:0), the results are riddled with errors. An additional factor that can contribute to the FID signal reduction is the presence of heteroatoms in a molecule. In the case of esters, a carbon atom is already oxidized in the starting sample. The oxidation energy leads to ionization, so in these molecules, the oxidized carbon atom is split, and this fragment cannot produce ions and a response in the detector. When this happens, it is necessary to use a response factor relative to each analyte with respect to an internal standard to correct the responses of the detector [13,18,19]. The experimental response factors (ERFs), theoretical response factors (TRFs), and error factors (EFs) used here were described in our previous study [13], and are listed in Table S1.

The precision of the quantitative methods was evaluated using intra-day repeatability and inter-day reproducibility experiments. Intra-day repeatability was determined from six complete analyses of each sample under the same conditions in one day. Inter-day reproducibility was determined from three complete analyses of each sample repeated on three consecutive days. Both intra-day and inter-day precision levels were satisfactory. The intra-day relative standard deviation (RSD) ranged from 0.4 to 3.8% and the inter-day RSD ranged from 0.5 to 7.6% (Table S1).

2.2. Comparison of Preparation Methods for FAME Determination

In the literature [20–25] and international standards [26], different methods have been proposed for the determination of FA profiles in cheese, mainly based on chromatographic analysis. Due to the 'complexity' of the matrix that is cheese, each procedure involves multiple steps. This can lead to analytical errors, resulting in a lack of reproducibility and unreliable results. Typically, the procedure for determining FA profiles in cheese follows three distinct steps: fat isolation, transesterification, and FA extraction. We selected several sample preparation methods that differed from each other to conduct our study, while the chromatographic analyses were carried out identically. One method that we did not use for our study is the one presented in ISO 14156, in which the fat is isolated using a Soxhlet extractor, because this method is very time-, labor-, and energy-intensive, and also involves considerable amounts of toxic solvents (more than 250 mL/sample). The Folch method, in which a chloroform–methanol mixture is used for fat isolation, is the most commonly applied procedure for FA determination. Many modifications have been introduced to this method to increase the efficiency of lipid isolation. Other methods of determining FA profiles in cheese are also used, in which safer solvents or solvent mixtures replace the toxic chloroform.

Our study selected six cheese sample preparation methods to determine the FA profile. The methods we chose varied in terms of the amount of reagents used, time consumption, and labor intensity. Three of these were the modified variants of the Folch method (D, E, and F). Each of the three Folch methods used different modifications to the original Folch method [27]. These modifications involved using different weighed amounts of cheese and different volumes of the chloroform–methanol mixture (2:1, *v*:*v*). In addition, in order to increase the efficiency of fat isolation, in Method E, an antioxidant (BHT) was added to the sample, and ultrasonication was applied, while in Method F, the resulting chloroform and aqueous layers were left for 24 h for more efficient/effective phase separation. In all the Folch-modified methods, the same amount of fat (100 mg) for the transesterification step was used. In the case of Method C, conversely to the Folch method, methanol and dichloromethane were used to isolate the fat, and all the extracted fat was trans-esterified. In addition, like Method E, Method C employed an antioxidant. The procedures of Methods A and B differed significantly from the others. It is presumed that their development aimed to identify a shortened/simplified and less solvent-intensive method of preparing cheese samples for analysis purposes. In Method A, the fat was not isolated from the cheese sample but was extracted directly with n-hexane, and the transesterification reaction was

carried out at the same time. In contrast, in Method B, ammonia, ethanol, and n-hexane were added to the cheese sample in the first step, and ethanol and n-hexane were then added twice.

Table 1 shows the FA profiles produced using Methods A–F. Unfortunately, no comparable study results were obtained. This is most evident for Methods A, B, and C ($p > 0.05$). The percentage of short-chain FAs (C4:0–C11:0) was very low in Methods A and B, while their content was highest in Method C. However, it was noted that for Method C, the resolution of chromatographic peaks deteriorated after several chromatographic analyses (about twenty). This indicated that there was contamination in the chromatographic system; sediment was found in the liner, pre-column, and gold seal (Figure S2). For the other methods, no such contamination was observed. This problem may have been due to the difference in the fat isolation from the cheese sample. Namely, Method C used methanol and dichloromethane for fat isolation. In the final step of this procedure, a derivatization reagent was added to the total fat obtained. This may have influenced the poorly selective isolation of fat from the cheese sample, resulting in other matrix components, i.e., protein, passing into the extract.

Table 1. Contents of fatty acids determined in cheese using different sample preparation methods (mean values in g/100 g FA ± SD).

Nr of FA	FA	Method A	Method B	Method C	Method D	Method E	Method F
1	C4:0	0.01 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}	4.80 ± 0.72 ^{c}	2.72 ± 0.24 ^{b}	2.97 ± 0.27 ^{b}	2.87 ± 0.39 ^{b}
2	C6:0	0.01 ± 0.00 ^{a}	0.09 ± 0.02 ^{a}	1.93 ± 0.29 ^{b}	1.41 ± 0.31 ^{bc}	1.13 ± 0.34 ^{c}	1.23 ± 0.39 ^{c}
3	C8:0	0.05 ± 0.04 ^{a}	0.27 ± 0.01 ^{a}	1.54 ± 0.10 ^{c}	1.08 ± 0.20 ^{b}	0.81 ± 0.23 ^{b}	0.90 ± 0.36 ^{b}
4	C10:0	1.06 ± 0.15 ^{a}	0.51 ± 0.21 ^{a}	4.66 ± 0.13 ^{d}	2.70 ± 0.09 ^{b}	2.43 ± 0.21 ^{bc}	2.01 ± 0.52 ^{c}
5	C11:0	0.01 ± 0.00 ^{c}	0.11 ± 0.03 ^{ab}	0.11 ± 0.01 ^{ab}	0.10 ± 0.00 ^{ab}	0.07 ± 0.01 ^{a}	0.12 ± 0.05 ^{a}
6	C12:0	2.96 ± 0.18 ^{a}	1.42 ± 0.30 ^{d}	4.98 ± 0.13 ^{e}	3.91 ± 0.21 ^{b}	3.47 ± 0.24 ^{abc}	2.94 ± 0.53 ^{ac}
7	C13:0	0.10 ± 0.00 ^{a}	0.49 ± 0.13 ^{b}	0.16 ± 0.01 ^{a}	0.15 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.12 ± 0.03 ^{a}
8	C14:0	12.07 ± 0.40 ^{a}	8.83 ± 0.86 ^{d}	13.94 ± 0.30 ^{e}	12.17 ± 0.58 ^{ab}	11.68 ± 0.45 ^{abc}	11.82 ± 1.54 ^{abc}
9	C14:1	1.20 ± 0.06 ^{ab}	0.96 ± 0.14 ^{ac}	1.75 ± 0.06 ^{d}	1.39 ± 0.07 ^{bde}	1.30 ± 0.04 ^{bcef}	1.30 ± 0.33 ^{bcef}
10	C15:0	1.95 ± 0.14 ^{a}	1.43 ± 0.04 ^{f}	2.21 ± 0.17 ^{abc}	2.28 ± 0.09 ^{cd}	1.96 ± 0.04 ^{be}	2.02 ± 0.28 ^{bde}
11	C15:1	0.41 ± 0.02 ^{a}	0.78 ± 0.36 ^{b}	0.51 ± 0.29 ^{abc}	0.37 ± 0.01 ^{ac}	0.32 ± 0.00 ^{ac}	0.36 ± 0.08 ^{ac}
12	C16:0	43.85 ± 1.33 ^{a}	37.17 ± 0.23 ^{b}	38.17 ± 1.22 ^{b}	41.62 ± 1.12 ^{ac}	42.45 ± 0.63 ^{ac}	41.94 ± 1.87 ^{ac}
13	C16:1	2.15 ± 0.10 ^{ab}	2.56 ± 0.03 ^{c}	2.21 ± 0.09 ^{ad}	2.42 ± 0.07 ^{c}	2.18 ± 0.03 ^{bde}	2.15 ± 0.12 ^{bde}
14	C17:0	0.98 ± 0.06 ^{a}	0.86 ± 0.12 ^{a}	0.90 ± 0.20 ^{a}	0.99 ± 0.03 ^{a}	0.93 ± 0.02 ^{a}	0.98 ± 0.12 ^{a}
15	C17:1	0.45 ± 0.01 ^{a}	0.70 ± 0.16 ^{b}	0.57 ± 0.24 ^{abc}	0.46 ± 0.05 ^{acd}	0.41 ± 0.01 ^{acde}	0.48 ± 0.06 ^{acde}
16	C18:0	8.29 ± 0.66 ^{b}	9.00 ± 1.35 ^{b}	4.56 ± 0.09 ^{c}	6.41 ± 0.28 ^{a}	6.62 ± 0.24 ^{a}	6.47 ± 0.29 ^{a}
17 + 18	C18:1n9t + C18:1n9c	19.60 ± 1.46 ^{b}	23.80 ± 0.59 ^{c}	12.76 ± 0.16 ^{d}	15.43 ± 1.12 ^{a}	16.66 ± 0.56 ^{a}	15.36 ± 1.67 ^{a}
19	C18:2n6c	0.65 ± 0.05 ^{a}	0.93 ± 0.33 ^{b}	0.54 ± 0.06 ^{a}	0.71 ± 0.02 ^{ab}	0.67 ± 0.04 ^{a}	0.45 ± 0.37 ^{a}
20	C18:2n6t	1.99 ± 0.08 ^{a}	2.67 ± 0.23 ^{d}	1.46 ± 0.04 ^{b}	1.68 ± 0.07 ^{bc}	1.65 ± 0.05 ^{bc}	1.75 ± 0.26 ^{ac}
21	C20:0	0.17 ± 0.02 ^{b}	0.28 ± 0.07 ^{a}	0.28 ± 0.09 ^{a}	0.28 ± 0.01 ^{a}	0.23 ± 0.02 ^{a}	0.21 ± 0.08 ^{a}
22	C18:3n3	1.11 ± 0.08 ^{a}	5.24 ± 0.68 ^{b}	1.03 ± 0.06 ^{a}	1.07 ± 0.04 ^{a}	1.11 ± 0.04 ^{a}	1.11 ± 0.07 ^{a}
23	C20:1n9	0.92 ± 0.02 ^{a}	1.84 ± 0.31 ^{c}	0.92 ± 0.07 ^{a}	1.12 ± 0.05 ^{ab}	1.13 ± 0.05 ^{ab}	1.23 ± 0.17 ^{b}
				Sums			
∑SFA [1]		71.52	60.53	78.25	75.82	74.87	73.62
∑UFA [2]		24.73	30.64	18.71	21.19	21.99	20.88
∑MUFA [3]		3.74	8.84	3.04	3.46	3.43	3.31
∑PUFA [4]		20.25	24.73	13.30	16.15	17.32	15.80

[1] ∑SFA = sum of saturated fatty acids; [2] ∑UFA = sum of unsaturated fatty acids; [3] ∑MUFA = sum of monounsaturated fatty acids; [4] ∑PUFA = sum of polyunsaturated fatty acids. Mean values with similar letters within a row are statistically similar, while mean values with different letters within a row are significantly different ($p < 0.05$).

The highest convergence for most acids ($p < 0.05$) was found for the three modified variants of the Folch method (D–F). Different modifications in the Folch method did not

affect the differences in the FA profile of the cheese sample. Still, differences in the intensity of the chromatographic peaks could be observed. Compared to Method D, Methods E, and F produced higher-intensity FA peaks (Figure S1). In Method E, the higher peak intensity, or extraction efficiency, may have been due to the use of an antioxidant and ultrasonication. The additional sonication may have contributed to increased diffusion between the cheese sample and the chloroform–methanol mixture. In Method F, the increase in extraction efficiency may have been influenced by the fact that the mixture of aqueous and chloroform phases was left until the following day, resulting in an effective separation of the two.

Given the comparable results produced by the modified variants of the Folch method (D–F), it can be concluded that they were the most reliable. In addition, the results of the FAs content in the Gouda-type cheese sample obtained by modified Folch methods are similar to the data presented in the literature [28–30]. This confirms the use of the Folch method as a reference method. Of the three modification of the Folch methods investigated, Method D was the most favorable due to the lowest time consumption and the least amount of stages.

2.3. Assessment of the Method of Greenness

This study assessed the 'green' nature of the compared analytical methods for operators and the environment, including solvent characterization, experiment time, energy consumption, and others. Two different matrices were used, the Analytical Eco-Scale tool, which is based on scoring (numerical assessment), and the Analytical Greenness Metric for Sample Preparation (AGREEprep) tool, which combines a graphical representation of the results with scoring.

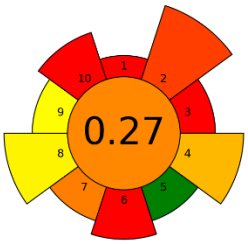
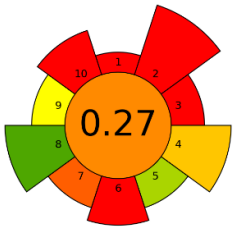
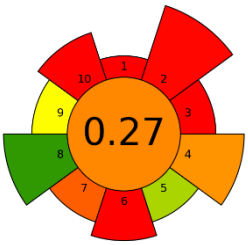
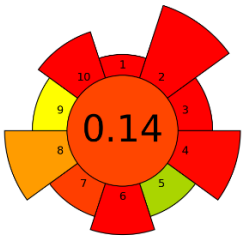
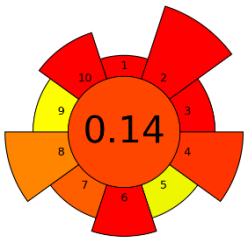
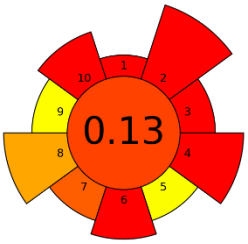
The Analytical Eco-Scale score was calculated by subtracting penalty points (PPs) from a base score of 100 for any factor in the analytical procedure, such as reagent quantity, hazard, energy consumption, and waste production. Green analysis was deemed ideal if it had an eco-scale value of 100, excellent if >75, acceptable if >50, and inadequate if <50 [31,32].

The AGREEprep tool was first introduced in 2022 [16]. This metric tool gives prominence to the sample preparation step of the analysis. The AGREEprep tool includes software (free website link) that generates a pictogram showing the performance of the method [33]. This metric tool is based on ten effect categories, which are recalculated into sub-scores on a 0–1 scale. The color of each section ranges from red to green. The categories include, but are not limited to, hazardous reagent consumption, waste generation, sample volume, throughput, and energy consumption. The evaluation produces a pictogram that summarizes the overall greenness of the method. The different parts of the pictogram allow the identification of weak and strong points of the method and provide a quick comparison of different methods [16]. The evaluation results of the methods used to analyze the FA profiles are shown in Table 2.

The AGREEprep tool more rigorously evaluated the procedures used, with summary pictograms colored orange to red and numerical scores ranging from 0.13 to 0.27. It can be seen that the first higher-scored group of procedures comprised Methods A, B, and C, which received the same total score of 0.27. The second group of Methods—D, E, and F—received lower scores of 0.14 (for Methods D and E) and 0.13 for (Method F).

A more focused evaluation of the first three methods was mainly influenced by criteria 5 and 8, which relate to a smaller sample size (0.05–1 g) and lower energy consumption. However, Methods D–F used time-consuming and energy-intensive steps, i.e., homogenization, ultrasonication, and centrifugation. The other evaluation parameters are comparable for all methods. Detailed method evaluation reports using the AGREEprep tool are presented in the Supplementary Materials.

Table 2. The greenness profile of the employed (A–F) methods for FA analysis in cheese samples using Eco-Scale and AGREE-prep metrics.

Method	Metrics	
	Analytical Eco-Scale Score	**AGREEprep Pictogram**
A	73 acceptable green analysis	
B	63 acceptable green analysis	
C	71 acceptable green analysis	
D	61 acceptable green analysis	
E	63 acceptable green analysis	
F	61 acceptable green analysis	

In the Analytical Eco-Scale assessment, all six methods scored 'acceptable green analysis' with scores ranging from 61 to 73. Method A received the highest score, while Methods D and F received the lowest score. Details of the input data used to assess each subcategory in Table S2 are provided in the Supplementary Materials.

The two scoring methods used to evaluate the methods used are not consistent with the authors' assessment. Our observations clearly show that Method F was the least favorable in all respects. This assessment is due to the high workload of the analyst and the sample preparation time of about 24 h; in addition, the procedure consisted of the largest number of steps (about 20). Sample preparation required 155 mL of hazardous materials (n-hexane, methanol, and KOH), resulting in large amounts of waste. However, this parameter (criterion 2 for the AGREEprep tool) scored similarly across all procedures, ranging from 0.13 (A) to 0.0 (B–F). This is due to the fact that sample preparation methods that used more than 10 mL or 10 g of hazardous solvents and reagents had a score of 0 on this rule. Similarly, to assess the amount of waste generated (criterion 4), Method E generated approx. 26 mL of waste and received a score of 0.1, while Method F generated approx. 300 mL of waste and received a similar score of 0.0.

Therefore, it can be concluded that the greenness assessment methods of sample preparation for analysis purposes are not adequate for comparing (classic) techniques such as those presented in this study. Greenness assessment methods help compare micro-extraction, automated, solvent-free, or low-solvent consumption methods.

3. Materials and Methods

3.1. Cheese Sample

The experimental material was commercial Gouda cheese, produced using cow milk from a Polish producer (Pomerania, Poland). The cheese was cut into smaller sections, vacuum-packed, and frozen at −18 °C. All experiments were performed using a fresh-opened cheese sample after it was defrosted at room temperature and ground it into small pieces using a cheese grater. The same cheese sample was used for all methods examined in this article.

3.2. Chemical and Reagents

Solvents and chemicals used for lipid extraction and FAME preparation were of analytical grade. Chloroform, dichloromethane, methanol, n-hexane, ethanol, hydrochloric acid, ammonia, anhydrous sodium sulfate, butylated hydroxytoluene (BHT), potassium chloride, sodium chloride, potassium hydroxide, sodium hydroxide, and hexadecane (used as internal standards) were purchased from Sigma Aldrich (Darmstadt, Germany). The standard mix of 37 FAMEs and the standard mix of C4:0–C24:0 saturated FAMEs were purchased from Supelco (Bellefonte, PA, USA).

3.3. Lipid Extraction and FAME Preparation

In this study, six different sample preparation methods were tested. The procedures were carried out as follows:

- Method A [20]: 50 mg of cheese was mixed with 1 mL of n-hexane and 0.2 mL of KOH/MeOH (0.2 M). The sample was vortexed for 3 min and allowed to rest for 15 min, and 1 mL of HCl/MeOH (10%) was added. The sample was vortexed for 10 s and incubated (50 °C/10 min). After cooling, 2 mL of ultrapure water and 2 mL of n-hexane were added to the sample. The sample was mixed for 10 s and centrifuged for 5 min, and then a 0.5 g of anhydrous sodium sulfate was added. The sample was vortexed for 30 s and centrifuged for 5 min. The n-hexane phase was collected. The total time of sample preparation was about 60 min.
- Method B [21]: 1 g of cheese was added to 0.4 mL of ammonia (25%), 1 mL of EtOH (95%), and 5 mL of n-hexane. After centrifugation, the upper layer was collected and the sample was re-extracted with 1 mL of EtOH (95%) and 5 mL of n-hexane. The sample was centrifuged, the upper layer was collected, and the sample was extracted again using 5 mL of n-hexane. All the obtained phases were collected. The upper phases obtained during each extraction were pooled together, dried under nitrogen, and dissolved in 1 mL of n-hexane. The total time of sample preparation was about 60 min.

- Method C [22]: 1 g of cheese was mixed (250 rpm; 3 min) with 4 mL of MeOH, 2 mL of dichloromethane, and 1 mg of BHT. Then, 2 mL of dichloromethane and 2 mL of distilled water were added to the sample and gently shaken for 20 s, followed by centrifugation (2800 rpm; 15 min). The apolar layer was collected in a glass vial, and the sample was evaporated with nitrogen (40 °C; 25 min). Afterward, 100 µL of KOH/MeOH (0.2 M) was added to the sample. The mixture was incubated (95 °C; 20 min) and cooled to stop the derivatization reaction, and 1 mL of n-hexane was added. The total time of sample preparation was about 90 min.
- Method D [23]: 1 g of cheese was homogenized in 15 mL of chloroform–methanol (2:1; v/v). The mixture was shaken mechanically (20 min) and centrifuged (7300 rpm; 5 min). Then, the mixture was filtered, and then 15 mL of chloroform–methanol and 3 mL of KCl (0.74%) were added to the filtrates. After centrifugation (7300 rpm; 5 min), the chloroform layer was collected and mixed with 3 g of anhydrous sodium sulfate. Then, the mixture was filtrated and the extract was concentrated by removing chloroform in a rotary evaporator and dried over a gentle stream of nitrogen. Then, 100 mg of the obtained fat was weighed in a test tube and dissolved in 5 mL of n-hexane. Next, 0.2 mL of KOH/MeOH (0.2 M) was added to the mixture and shaken vigorously with a vortex mixer (1 min). After an additional reaction time of 5 min, 0.5 g of anhydrous sodium hydrogen sulfate was added and mixed again. The sample was centrifuged (3 min) and the extract was collected. The total time of sample preparation was about 95 min.
- Method E [24]: 2.5 g of cheese was added to a 25 mL of chloroform–methanol (2:1; v/v) and BHT (0.001%). The mixture was homogenized (2500 rpm; 30 min) and ultrasonicated (Amplifier 35%; 20 min), and 10 mL of saturated NaCl solution was added. The suspension was then centrifuged (20 min; 4000 rpm). The chloroform layer was removed using a rotary evaporator. Then, 100 mg of the obtained fat was weighed in a test tube and dissolved in 5 mL of n-hexane. Next, 0.2 mL of KOH/MeOH (0.2 M) was added to the mixture and shaken vigorously with a vortex mixer (1 min). After an additional reaction time of 5 min, 0.5 g of anhydrous sodium hydrogen sulfate was added and mixed again. The sample was centrifuged (3 min) and the extract was collected. The total time of sample preparation was about 100 min.
- Method F [25]: The 3 g samples were homogenized (1 min) with 30 mL of MeOH. Then, 30 mL of chloroform was added, and the mixture was homogenized (2 min). The prepared mixture was filtered into a glass cylinder. The solid residue was mixed in 60 mL of chloroform–methanol (2:1; v/v) and homogenized again for 3 min. The mixture was transferred to the same cylinder. Next, NaCl (0.88%) in water was added to the total filtrate (in the amount constituting $\frac{1}{4}$ of the filtrate volume), then shaken and left overnight. The lower layer was mixed with H_2O/MeOH (1:1; v/v). The washing procedure was repeated. The remaining layer was dehydrated with anhydrous sodium sulfate and the mixture was evaporated. Then, 100 mg of the obtained fat was weighed in a test tube and dissolved in 5 mL of n-hexane. Next, 0.2 mL of KOH/MeOH was added to the mixture and shaken vigorously with a vortex mixer (1 min). After an additional reaction time of 5 min, 0.5 g of anhydrous sodium hydrogen sulfate was added and mixed again. The sample was centrifuged (3 min) and the extract was collected. The total time of sample preparation was about 26 h.

3.4. GC Analysis

The FA profile identification and quantification processes were performed using a gas chromatograph (Agilent 7890B, Santa Clara, CA, USA), equipped with a flame ionization detector (FID) and a fused silica capillary SP-2380 column (30 m × 0.25 mm × 0.2 µm) (Supelco, Bellefonte, PA, USA), with a constant flow of 1.0 mL/min helium as the carrier gas.

The injector port was held at 230 °C and used in the split mode using a split ratio of 10:1, and injection volumes were 1 µL. The detector temperature was 250 °C. The GC oven

temperature program started at 50 °C and increased to 220 °C at 4 °C/min where it was held for 15 min.

3.5. Statistical Analysis

Peak areas were obtained by manual integral with Agilent ChemStation F.01.00.1903 (Agilent Technologies, Santa Clara, CA, USA). The experiments were performed in triplicate and the analysis was repeated at least three times. The results were expressed as the mean ± standard deviation. The data were subjected to a one-way analysis of variance (ANOVA) and Tukey's test. Results were considered statistically significant at $p < 0.05$.

4. Conclusions

The methodologies chosen to extract the lipid fraction from the cheese matrix may differently affect the yield of extracted lipids. In this study, six analytical methods were used to determine the FA profile of the cheese sample. In order to simplify the selection of the method used for the routine analyses of the FA profile in cheese, an assessment of the environmental impact of these methods was used. To this end, the Analytical Eco-Scale tool and the Analytical Greenness Metric for Sample Preparation (AGREEprep) tool were applied.

The results showed significant differences ($p > 0.05$) in the content of the individual acids obtained using the different methods. It was observed that the results generated by the three modified Folch method variants showed the highest similarity and the least significant differences ($p < 0.05$). In contrast, the other three methods showed a large discrepancy between the results ($p > 0.05$). However, Methods A, B, and C obtained better scores on the assessment of greenness than the modified Folch methods.

This study shows that it is important to compare different methodologies for the determination of fatty acids in complex matrices such as cheese. The use of greenness tools allows the suitability of these analytical procedures to be assessed in terms of environmental friendliness. In addition, an assessment of analytical methods results in significant economic benefits (costs of reagents and waste, time and energy reductions, etc.) and will positively affect the health of operators.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules28134981/s1. Figure S1: Chromatograms obtained Methods A, B, C, D, E, and F. Figure S2: Inlet gold seal and inlet liner after chromatographic analysis of samples (about 20) made using Method C. Table S1: Experimental and theoretical correction factors, error factor, and intra-day and inter-day precision for the fatty acid in cheese sample. Table S2: Calculated PPs (Eco-Scale) for evaluated analytical procedures for FA determination in cheese samples (Methods A–F). Reports from the AGREEprep tool for Methods A–F.

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Supplementary Materials

A Comparative Analysis on the Environmental Impact of Selected Methods for Determining the Profile of Fatty Acids in Cheese

Table S1. Experimental and theoretical correction factors, error factor, intra-day and inter-day precision for the fatty acid in cheese sample.

Fatty acid		ERF [1]	TRF [2]	EF [3]	Intra-day RSD (%)	Inter-day RSD (%)
butyric acid	C4:0	1.2144	1.5742	0.7714	3.8	3.3
caproic acid	C6:0	1.0371	1.3378	0.7752	3.1	1.0
caprylic acid	C8:0	1.0509	1.2195	0.8617	2.9	1.9
capric acid	C10:0	1.0681	1.2702	0.8408	1.4	1.5
undecanoic acid	C11:0	1.0552	1.1486	0.9186	2.6	1.6
lauric acid	C12:0	1.0614	1.1013	0.9637	2.3	3.7
oleic acid	C13:0	1.0516	1.0831	0.9709	3.1	4.0
myristic acid	C14:0	1.0432	1.0675	0.9772	2.1	3.9
myristoleic acid	C14:1	1.0372	1.0587	0.9796	2.5	5.1
pentadecylic acid	C15:0	1.0226	1.0540	0.9702	2.6	2.6
ginkgolic acid	C15:1	1.0211	1.0457	0.9764	2.3	7.6
palmitic acid	C16:0	0.9816	1.0422	0.9418	2.2	3.3
palmitoleic acid	C16:1	1.0008	1.0345	0.9674	1.7	1.3
heptadecanoic acid	C17:0	0.9920	1.0318	0.9614	0.3	2.1
10-heptadecenoic acid	C17:1	0.9953	1.0244	0.9715	0.6	3.3
stearic acid	C18:0	0.9659	1.0225	0.9446	0.8	2.1
elaidic acid + oleic acid	C18:1n9t + C18:1n9c	0.9630	1.0155	0.9483	0.9	2.0
linolealidic acid	C18:2n6c	0.9814	1.0087	0.9729	0.7	2.2
linoleic acid	C18:2n6t	0.9883	1.0087	0.9797	0.9	2.5
arachidic acid	C20:0	0.9813	1.0067	0.9747	0.5	2.0
alpha-linolenic acid	C18:3n3	0.9821	1.0017	0.9804	0.4	0.5
11-eicosenoic acid	C20:1n9	1.4001	1.0005	1.3994	1.9	1.5

[1] ERF = Experimental response factor, [2] TRF = Theoretical response factor, [3] EF = Error factor (ERF/TRF).

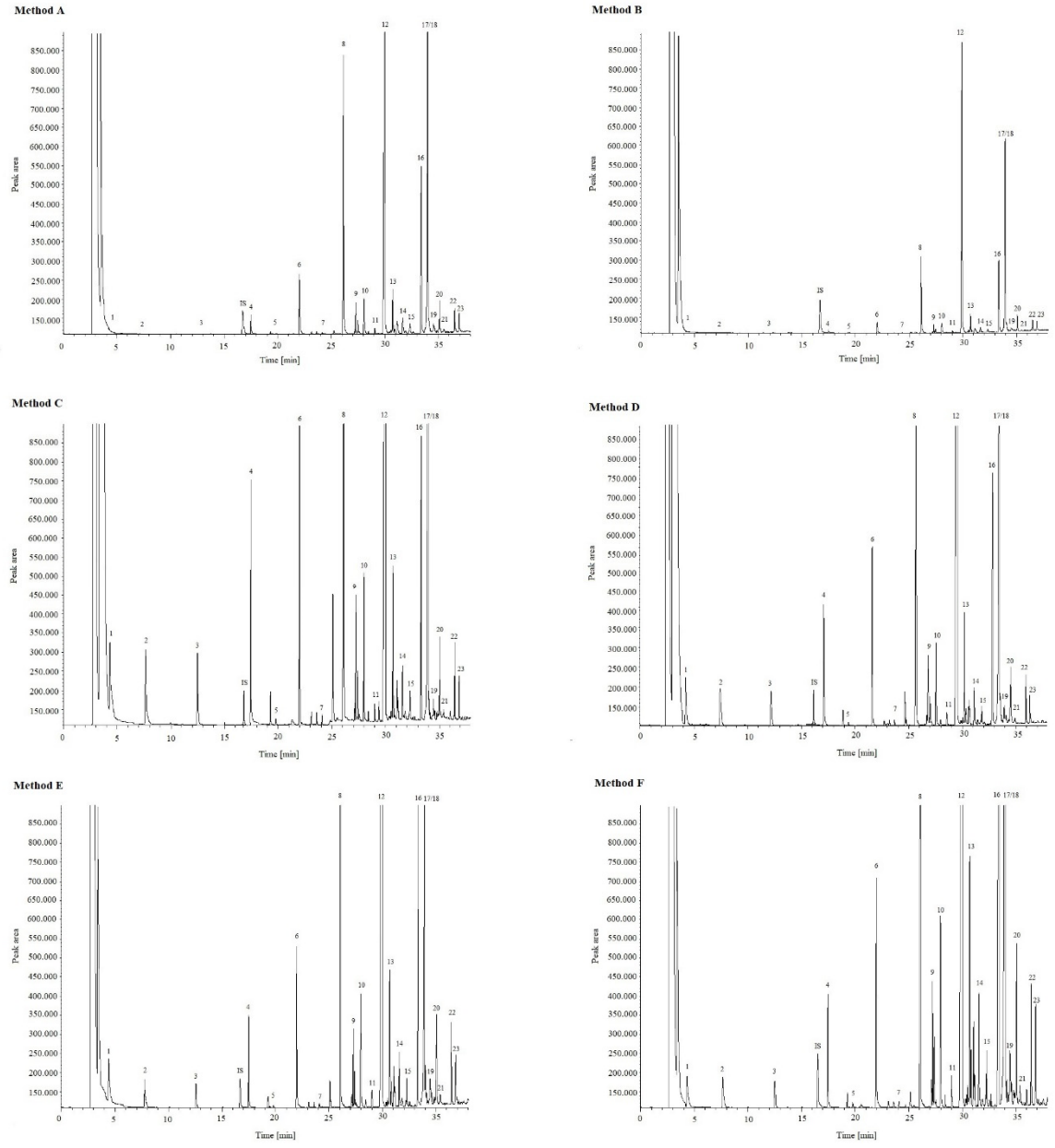

Figure S1. Chromatograms obtained A, B, C, D, E, and F methods.

Figure S2. Inlet gold seal and inlet liner after chromatographic analysis of samples (about 20) made using the C method.

Table S2. Calculated PPs (Eco-Scale) for evaluated analytical procedures for FAs determination in cheese samples (Procedures A - C).

Procedure A	
Reagents	**PPs**
Methanol: 0.1 mL	6
Na_2SO_4(aq.): 0.5 g	0
KOH: 0.1 g	2
n-hexan: 4 mL	8
	∑16
Instrument	**PPs**
Transport	1
Energy (≤0.1 kWh)	0
Occupational hazard	3
GC-FID	1
Waste (1-10 mL, no treatment)	6
	∑11
Total PPs	**27**
Score – acceptable green analysis	**73**

Procedure B	
Reagents	**PPs**
Ethanol: 2 mL	2
n-hexan: 16 mL	16
NH_3 (aq.): 0.4 mL	6
	∑ 24
Instrument	**PPs**
Transport	1
Energy (≤0.1 kWh)	0
Occupational hazard	3
GC-FID	1
Waste (>10 mL, no treatment)	8
	∑13
Total PPs	**37**
Score – acceptable green analysis	**63**

Procedure C	
Reagents	**PPs**
Methanol: 4.1 mL	6
KOH: 0.05 g	2
n-hexan: 1 mL	8
Dichloromethane: 4 mL	2
	∑ 18
Instrument	**PPs**
Transport	1
Energy (≤0.1 kWh)	0
Occupational hazard	3
GC-FID	1
Waste (1-10 mL, no treatment)	6
	∑11
Total PPs	**29**
Score – acceptable green analysis	**71**

Table S2 con. Calculated PPs (Eco-Scale) for evaluated analytical procedures for FAs determination in cheese samples (Procedures D - F).

Procedure D	
Reagents	**PPs**
Chloroform: 20 mL	4
Methanol: 10.1 mL	12
Na_2SO_4: 3.5 g	0
KCl (o.74%): 3 g	0
n-hexan: 5 mL	8
KOH: 0.1 g	2
	∑26
Instrument	**PPs**
Transport	1
Energy (≤0.1 kWh)	0
Occupational hazard	3
GC-FID	1
Waste (>10 mL, no treatment)	8
	∑13
Total PPs	**39**
Score – acceptable green analysis	**61**

Procedure E	
Reagents	**PPs**
Chloroform: 16 mL	4
Methanol: 11 mL	12
NCl (aq): 15 mL	0
NaOH: 0.2 g	2
Isooctane: 3.5 mL	8
Na_2SO_4: 2 g	0
	∑ 26
Instrument	**PPs**
Transport	1
Energy (≤1.5 kWh)	1
Occupational hazard	0
GC-FID	1
Waste (>10 mL, no treatment)	8
	∑11
Total PPs	**37**
Score – acceptable green analysis	**63**

Procedure F	
Reagents	**PPs**
Methanol: 80 mL	12
Chloroform: 70 mL	4
NCl (aq): 30 mL	0
Na_2SO_4: 0.5 g	0
KOH: 0.05 g	2
n-hexan: 5 mL	8
	∑ 26
Instrument	**PPs**
Transport	1
Energy (≤0.1 kWh)	0
Occupational hazard	3
GC-FID	1
Waste (>10 mL, no treatment)	8
	∑13
Total PPs	**39**
Score – acceptable green analysis	**61**

AGREEprep
Analytical Greenness Metric for
Sample Preparation

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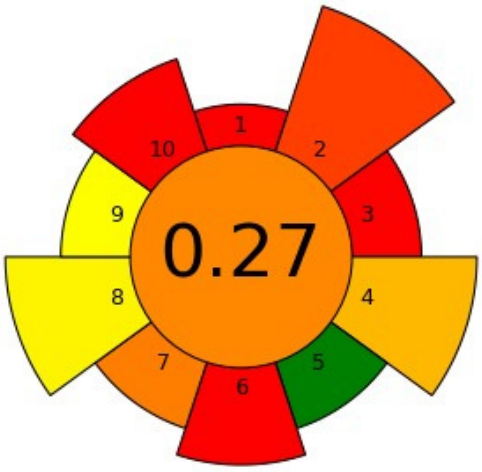

Method A

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 4.2	0.13	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 5.2	0.36	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 0.05	1.0	2
6.	**Sample throughput** Hourly sample throughput: 1	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 2 steps or fewer; degree if automation: Manual systems	0.25	2

8.	**Energy consumption** Approximate energy consumption per analysis [W]: 76	0.48	4

9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	2

10.	**Operator's safety** No. of distinct hazards: 4 or more hazards	0.0	3

AGREEprep
Analytical Greenness Metric for
Sample Preparation

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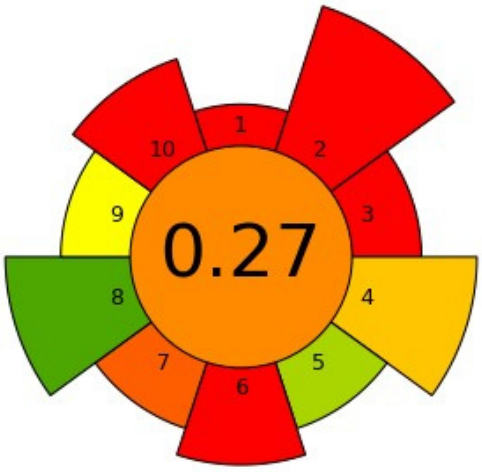

Method B

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 18.4	0.0	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 4.4	0.39	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 1	0.67	2
6.	**Sample throughput** Hourly sample throughput: 1	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 3 steps; degree if automation: Manual systems	0.19	2

8.	**Energy consumption** Approximate energy consumption per analysis [W]: 18	0.85	4

9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	2

10.	**Operator's safety** No. of distinct hazards: 4 or more hazards	0.0	3

AGREEprep
Analytical Greenness Metric for
Sample Preparation

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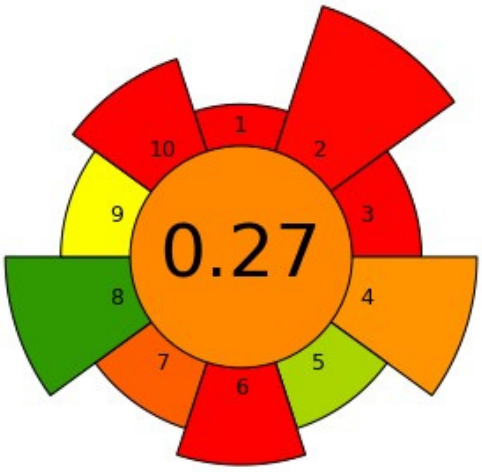

Method C

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 9.1	0.01	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 8.1	0.29	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 1	0.67	2
6.	**Sample throughput** Hourly sample throughput: 0.66	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 3 steps; degree if automation: Manual systems	0.19	2

8.	**Energy consumption** Approximate energy consumption per analysis [W]: 14.3	0.91	4

9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	2

10.	**Operator's safety** No. of distinct hazards: 4 or more hazards	0.0	3

AGREEprep
Analytical Greenness Metric for
Sample Preparation

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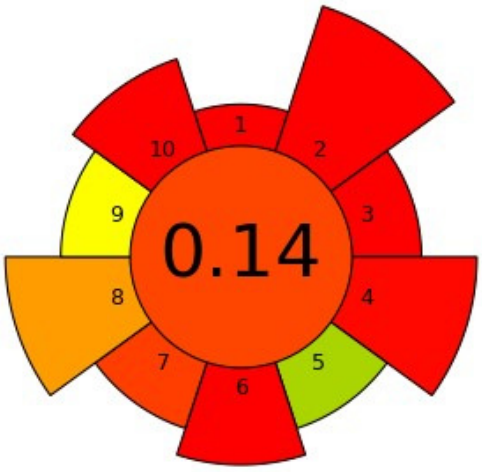

Method D

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 35.2	0.0	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 44.7	0.02	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 1	0.67	2
6.	**Sample throughput** Hourly sample throughput: 0.6	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 4 steps; degree if automation: Manual systems	0.12	2

8.	**Energy consumption** Approximate energy consumption per analysis [W]: 148	0.31	4

9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	2

10.	**Operator's safety** No. of distinct hazards: 4 or more hazards	0.0	3

AGREEprep
Analytical Greenness Metric for
Sample Preparation

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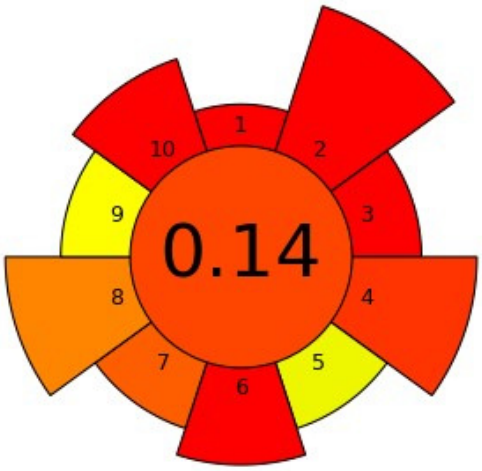

Method E

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 35.2	0.0	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 26.2	0.1	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 2.5	0.53	2
6.	**Sample throughput** Hourly sample throughput: 0.6	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 3 steps; degree if automation: Manual systems	0.19	2

8.	**Energy consumption** Approximate energy consumption per analysis [W]: 178	0.26	4

9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	2

10.	**Operator's safety** No. of distinct hazards: 4 or more hazards	0.0	3

AGREEprep
Analytical Greenness Metric for
Sample Preparation

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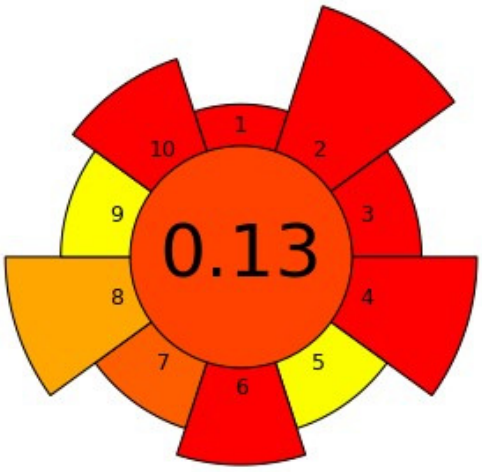

Method F

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 195.2	0.0	5
3.	**Sustainability and renewability of materials** < 25% of reagents and materials are sustainable or renewable and can only be used once	0.0	2
4.	**Waste** Mass [g] or volume [mL] of waste: 301.7	0.0	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 3	0.51	2
6.	**Sample throughput** Hourly sample throughput: 0.04	0.0	3
7.	**Integration and automation** No. of sample prep. steps: 3 steps; degree if automation: Manual systems	0.19	2

8.	**Energy consumption** Approximate energy consumption per analysis [W]: 138	0.33	4

9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	2

10.	**Operator's safety** No. of distinct hazards: 4 or more hazards	0.0	3

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Comparison of the Greenness Assessment of Chromatographic Methods Used for Analysis of UV Filters in Cosmetic Samples

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Abstract: Chemical ultraviolet filters are widely used in a variety of cosmetic products to protect the skin from the harmful effects of UV radiation. In order to guarantee consumers' health, the content in sunscreens is regulated in a number of countries. Many analytical methods are used to determine UV filters in cosmetics samples. In recent years, attention has been paid to the fact that the methods should have a small impact on the environment. This work examined the greenness of 10 reported chromatographic methods in the literature for the determination of UV filters in cosmetic samples using two new tools: analytical greenness metric (AGREE) and analytical greenness metric for sample preparation (AGREEprep). Microextraction methods of sample preparation in the AGREEprep assessment show a higher score of greenness. The results recommended the use of both tools to assess the greening of methods before planning laboratory analytical methods to measure their ecological impact on the environment.

Keywords: AGREE; AGREEprep; chromatography; greenness assessment; UV filters

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1. Introduction

UV filters are a group of chemicals commonly used in a wide range of cosmetic products (such as lotions, shampoos, creams, aftershave products, and make-up products) to protect the skin and hair from sun damage [1,2]. UV filters are classified into two groups: organic (chemical) UV filters, which absorb UV light, and inorganic (physical) UV filters, which reflect and scatter UV radiation. European legislation has set the maximum allowed concentration for each UV filter in cosmetic products (regulation no. 1223/2009 of the European Commission) [3]. The European Union regulations permit the use of 30 UV filters in cosmetics in concentrations ranging from 2 to 25%, of which only two are inorganic (titanium dioxide and zinc oxide). Chemical UV filters are organic molecules capable of absorbing high UV-A and UV-B range radiation [4]. They can be classified into the following different groups according to their chemical structure: benzophenone derivatives, p-aminobenzoic acid derivatives, salicylates, cinnamates, camphor derivatives, triazine derivatives, benzotriazole derivatives, benzimidazole derivatives, and others [5].

In order to ensure the product user's safety and to control the efficacy of sunscreen products, two standard methods are dedicated to the determination of 10 and 22 UV filters in sunscreen products [6,7]. Additionally, there are several dozen developed analytical methods for the determination of UV filters in cosmetic products in the research papers. However, there is still a need to develop a reliable, fast, and easy-to-implement analytical methodology for the analysis of cosmetic ingredients for the cosmetics industry, which, in accordance with current trends in analytical chemistry, should comply with the principles of green analytical chemistry (GAC) [5].

The analytical techniques employed to determine UV filters in cosmetics are gas and liquid chromatography with different detectors, spectroscopic techniques, and electrochemical techniques. The most common technique used for this purpose is liquid

chromatography, for which the extraction of analytes from a cosmetic product can be conducted with organic solvents, such as methanol or ethanol. Moreover, the use of an ultrasonic bath can accelerate the dissolution of these compounds. The samples can then be filtered or centrifuged to isolate the fraction of interest and remove the insoluble fraction of the cosmetic matrix. However, this method of sample preparation is not recommended because it may adversely affect the chromatographic system, causing damage to the injector, column, and detector [8]. Therefore, it is appropriate to use sample preparation techniques—selective extraction of analytes. Thanks to this, gas chromatography can also be used for analysis but sometimes requires a derivatization step (to increase the volatility and sensitivity of the compounds).

Recently, the most amount of attention has been paid to miniaturized extraction methods that comply with the GAC principles—aiming to offer safe analytical practices for humans and the environment. GAC was introduced to minimize the negative environmental impact of chemical measurements by reducing energy demand, toxic laboratory waste, and the use of hazardous solvents and chemicals. GAC has several metric tools that have been established to evaluate and measure the greening of methods by combining results (penalty points) or graphics such as the analytical eco-scale (AES), the green analytical procedure index (GAPI), the national environmental methods index (NEMI), the green certificate (GC), pictograms like hexagon (H), the analytical greenness metric (AGREE), and the analytical greenness metric for sample preparation (AGREEprep) [9–13]. Additionally, the concept of white analytical chemistry (WAC) is used to evaluate analytical methodologies, which is an extension of GAC [14].

Nowadays, the most popular free software metrics to assess the greenness of an approach are AGREE and AGREEprep. The AGREE metric tool is focused on the entire methodology and is based on the 12 categories of the principles of GAC, whereas the AGREEprep metric provides prominence to sample preparation in accordance with the 10 green sample preparation (GSP) principles.

The main task of this work is to assess the environmental impact of 10 chromatographic procedures for the analysis of UV filters in cosmetic samples using the latest tools, i.e., AGREE and AGREEprep. The use of assessment tools will highlight the advantages and disadvantages of each and indicate the most environmentally and operator-friendly methods. In addition, greenness assessment tools can help analysts identify critical steps in the chosen method so that the greenness of the analytical procedure can be improved. The results of this work can help scientists choose an analytical procedure for routine analysis.

2. Materials and Methods

2.1. Greenness Assessment Tools

Two new greenness assessment methods were applied in our study: AGREE and AGREEprep.

2.1.1. AGREE—Analytical Greenness Metric

AGREE was developed in 2020 by Pena-Pereira et al. [13]. The AGREE metric tool is focused on all analytical procedures and is based on the 12 categories of the principles of GAC, such as nature and volume of reagents, generated waste, energy consumption, the number of procedural steps, miniaturization, automation, and throughput (Table 1). Each input principle is transformed into a score range of 0–1, with weights for each principle, which are reflected in the width of each segment. The final score is obtained from the assessment of all the principles. The output is a clock-like pictogram where the final score and color are shown in the middle (from green to red). In this way, the environmental performance of the entire procedure can be easily assessed in terms of the 12 GAC principles [9]. The software is open-source and can be downloaded from https://mostwiedzy.pl/AGREE (accessed on 13 October 2023).

Table 1. Description of the criteria for AGREE and AGREEprep metrics.

AGREEprep Metric			AGREE Metric		
Criterion	**Criterion Description**	**Weight**	**Criterion**	**Criterion Description**	**Weight**
1	Favor in situ sample preparation	1	1	Sample pretreatment	2
2	Use safer solvents and reagents	5	2	Sample size	2
3	Target sustainable, reusable, and renewable materials	2	3	Location of the analytical device	2
4	Minimize waste	4	4	Number of steps	2
5	Minimize sample, chemical, and material amounts	2	5	Miniaturization and automatization	2
6	Maximize sample throughput	3	6	Derivatization agent	2
7	Integrate steps and promote automation	2	7	Waste amount	2
8	Minimize energy consumption	4	8	Analyte throughput	2
9	Choose the greenest possible post-sample preparation configuration for analysis	2	9	Energy consumption	2
10	Ensure safe procedures for the operator	3	10	Use of bio-based reagents	2
			11	Amount of toxic reagents	2
			12	No. of threats—operator's safety	2

2.1.2. AGREEprep—Analytical Greenness Metric for Sample Preparation

AGREEprep is a new analytical greenness metric, which was published by Wojnowski et al. [15] in 2022 and focuses on sample preparation for analysis. The assessment with AGREEprep is easy to perform for both inputting values and reading output. The free version of the software can be obtained from https://mostwiedzy.pl/AGREE (accessed on 13 October 2023). In this analytical greenness metric, each of the 10 criteria (Table 1) has a default weight that contributes to the total score. Researchers may make modifications to the default weights of each criterion, as long as they duly justify these changes. The assessment result is a colorful round pictogram with the number in the centre. The total score, which also ranges from 0 to 1, with 1 being the perfect score, is calculated by weighting the values from each criterion. If the cumulative result is greater than 0.5, it is considered a green method of analysis [16].

2.2. Evaluated Methods

Ten different analytical methods for the determination of UV filters in cosmetics were selected for evaluation of their greenness. Methods were selected that were based on commonly known and used analytical techniques. One of the procedures is a European standard (Method 1) [7], while the others are taken from literature reports. These procedures use various sample preparation methods, such as dissolving the cosmetic in a solvent (Methods 1 and 2) [7,17], solvent extraction with derivatization [18] (Method 3), SPE [19], and PLE [20] extraction methods (Methods 4 and 5), and five microextraction methods: MEPS (Method 6) [19], μ-MSPD (Method 7) [21], DSPME (Method 8) [22], US-VA-DLLME (Method 9) [23], and dynamic HF-LPME-HPLC-UV (Method 10) [24]. These methods use chromatographic techniques for analysis: gas chromatography with MS and MS/MS detectors and liquid chromatography with UV-Vis, DAD, and MS/MS detectors.

Descriptions of the analytical procedures of Methods 1–10 and detailed AGREEprep and AGREE reports are provided in Supplementary Materials.

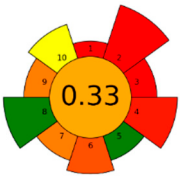
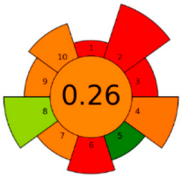
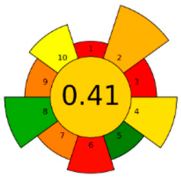
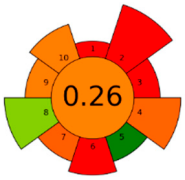
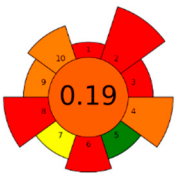
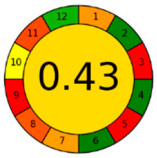
3. Results and Discussion

3.1. Comparison of Analytical Methodologies through the Greenness Assessment

Due to the complexity of the matrix of cosmetics, analytical procedures are time-consuming, energy-intensive, and often require the use of large amounts of solvents. Some analytical methodologies require the use of derivatization of analytes, which is harmful to the environment and, potentially, also to the health of the analyst. Because chromatography is the most widely used analytical method, procedures using this analytical technique were selected to assess their environmental impact. Ten methods were selected, including two

methods (4 and 6) previously described by the authors [19]. Five methodologies based on gas chromatography and five using liquid chromatography were considered in the assessment of greenness. The HPLC methods make it possible to perform analysis without the use of the analyte extraction step; these procedures require the ultrasonically assisted dissolution of cosmetics in solvents and filtration of the solutions. In addition, the content of UV filters in cosmetics is high (max. 25%), so the sample preparation procedures do not require a preconcentration of analytes. Therefore, liquid chromatography is the most commonly used analytical technique for the qualitative and quantitative determination of cosmetic ingredients. Two European standard methods [6,7] for the determination of UV filters in cosmetic samples use this analytical technique. One of them, with a higher "greenness" score (Method 1) is presented in the work. This procedure (EN 17156:2018) makes it possible to determine 22 UV filters. However, separate analyses should be performed for water-soluble UV filters (6 analytes), fat-soluble analytes (15 analytes), and one polymeric UV filter (Polysilicone-15). The procedure for determining the fat-soluble UV filters was selected for the "greenness" assessment due to the largest number of analytes. This method (Method 1), among the methods presented in Table 2, obtained a high AGREEprep score of 0.33. Sample preparation is simple and quick (30 min), which affects positively criterion 6 (*sample throughput*), moreover uses a small amount of energy (20 min ultrasonication, criterion 8). In this procedure, 32 mL of ethanol (EtOH) was used to dissolve the cosmetic sample. This large amount of reagent used for sample preparation results in a score of 0 in criterion 2 (*safer solvents and reagents*; red color), it is also associated with the generation of large amounts of waste (criterion 4, red color). Whereas, the EtOH, used for sample preparation, has only two pictograms, which is positively assessed in criterion 10 (*safe for operator*, yellow color). Compared to Method 1, Method 2 obtained a lower AGREEprep score 0.26. This is mainly due to the use of 10 mL of methanol (MeOH) (criterion 2), which has three pictograms, which have impact on reduce criterion 10 (orange color). In addition, the time of ultrasonication (90 min, criterion 6, red color) influenced the lowering of the score. Methods 1 and 2 use liquid chromatography for analysis, which is associated with the use of even greater amounts of solvents and generated waste. The AGREE scores for methods 1 and 2 were 0.48 and 0.43, respectively. These two highest scores (in Table 2) were obtained thanks to four criteria: the amount of the sample (0.1 g, criterion 2), the number of procedures steps (2 steps, criterion 4), no derivatization steps (criterion 6) and safety for the analyst (criterion 12). Compared to other presented procedures, Methods 1 and 2 use EtOH and MeOH to dissolve the samples, which has a positive effect on the assessment and improves the "greenness" of criterion 10 (*can be from bio-based sources*). As in the AGREEprep assessment, these methods are disadvantaged by the large amount of waste generated. The waste includes solvents, a filter, and a mobile phase used for HPLC analyses. The time needed to analyse one sample using Method 1 is long and takes to 70 min (*sample throughput*, criterion 8). This is due to the long-time of HPLC analysis (40 min). During this time, as many as 15 UV filters can be determined (light green color, criterion 8). However, Method 2, despite the short-term HPLC analysis (6 min, criterium 8), obtained a lower score due to the longer sample preparation time (ultrasonication—90 min) and low number of determined analytes (5 UV filters). The final low assessment of Method 2 is also affected by the high energy consumption associated with the long duration of ultrasonication but, above all, with the use of the MS/MS detector for the analysis (red color, criterion 9).

Table 2. The greenness results of methods 1–5 evaluated by AGREEprep and AGREE metrics.

Metric	Method 1 [7] Dissolution HPLC-UV-Vis	Method 2 [17] Dissolution HPLC-MS/MS	Method 3 [18] Solvent Extraction GC-MS/MS	Method 4 [19] SPE-GC/MS	Method 5 [20] PLE-GC-MS/MS
AGREEprep					
AGREE					

In Method 3, nail products were extracted with acetone, followed by vortexing, sonication, centrifugation, and filtering. During the extraction procedure, the UV filter was derivatized using *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide, and GC–MS/MS was used for analysis. This sample preparation procedure was evaluated by AGREEprep with a score of 0.41, the highest score among the methods presented in Table 2. Only 0.8 mL of acetone was used to extract analytes from a 0.1 g sample (green color, criterion 5), which favorably influenced the evaluation of criterion 2 (*use safer solvent* and *reagent*) and, thus, criterion 4 (*minimize waste*). The reagents used (acetone and derivatizing reagent) have only two pictograms, which has a positive effect on criterion 10 (yellow color). On the other hand, Method 3 received a low AGREE score of 0.39. Despite the high scores of criteria 7 and 11 (*waste amount* and *volume of toxic reagents*), criteria 6, 8, and 9 significantly lowered the final result of the procedure. The procedure presented by the authors of the paper focused on the determination of only one UV filter, and the time needed to perform one analysis was about 1 h, which resulted in a low evaluation of criterion 8 (*analyte throughput*). In addition, the low scores for parameters 6 and 9 were affected by the use of a derivatization agent and the use of the MS/MS (*energy consumption*) detector, respectively.

In Method 4, the authors used solid phase extraction (SPE), which is a classic, commonly used method of extraction, purification, and concentration of analytes. It achieved the same AGREEprep score as Method 2, 0.26. Only in a detailed report (see Supplementary Materials) can you notice slight differences in criteria 4, 6, 7, and 8. In this assessment, only two criteria were green: the amount of sample used (0.1 g, criterion 5) and the energy consumption needed to operate the vacuum pump and stirrer (criterion 8). However, this procedure is time-consuming (criterion 6) and consists of three steps: dissolving the cosmetic in water (15 min), extraction lasting approx. 25 min, evaporation of the solvent, and dissolution of the residue (20 min). This method uses large amounts of solvent (15.5 mL, criterion 2). Preparation of one sample generates 14.6 g of waste (criterion 4) consisting of solvents and cartridge SPE. Whereas, water (a cosmetic with water) is not added to the waste, because it is not mixed with toxic and harmful reagents. In addition, 5 mL of solvent is evaporated. On the other hand, the SPE-GC/MS method was assessed more critically by AGREE. The score was 0.38. The lowering of the score (red color) is due to the lack of miniaturization and automation (criterion 5), and high energy consumption—mainly through the use of GC/MS (criterion 9) and not the use of bio-based reagents (criterion 10).

The pressurized liquid extraction (PLE) followed by GC-MS/MS—Method 5—was applied for the simultaneous extraction and analysis of 16 UV filters from cosmetic and personal care products. Extraction was performed on an accelerated solvent extractor (ASE 150). The method was qualified as semi-automatic, which resulted in a higher score in criterion 7. However, the other criteria adversely affected the final score of AGREEprep, which is why this method received the lowest score of 0.19. Method 5 also resulted in the

lowest AGREE score, 0.38. Derivatization conducted for 60 min causes three criteria to be lowered simultaneously: use of derivatization agent, analyte throughput, and energy consumption. Also, conducting the analysis using GC-MS/MS significantly increases energy consumption, which further lowers the assessment of criterion 9 in AGREE metric.

Microextraction in packed sorbent (MEPS)—Method 6—is a greener alternative to the conventional SPE, which uses the same syringe for sample extraction and extract injection into the analytical instrument. In this method, smaller volumes of solvents and samples are used, and, as well, sample preparation takes less time than in classical SPE. MEPS can be performed online in a fully automated manner, but, due to high costs, equipment is most often purchased and the process conducted manually. The assessed MEPS procedure is a miniaturized version of the previously presented Method 3 (SPE), developed by the authors in this paper. The miniaturization of the procedure resulted in a significant improvement in the greenness score, which was achieved by AGREEprep with the highest score of 0.41. A satisfactory result is mainly influenced by four criteria—miniaturization (criterion 5), lower consumption of toxic solvents (criterion 2), smaller amount of generated waste (criterion 4), and significantly shortened sample preparation time (criterion 6). Criterion 3—sustainability and renewability of materials—was rated higher than the other assessed methods only in this method because the sorption bed is reusable. The evaluation of the entire procedure performed by AGREE was 0.48. It gained an advantage over other evaluated methods mainly in the criteria concerning the number of toxic reagents used (criterion 11) and the amount of generated waste (criterion 7). On the other hand, it is disadvantageous due to the use of an energy-intensive MS detector for the analysis (decrease in criterion 9). In addition, no bio-based reagents were used in the tests, which results in a negative assessment of criterion 10.

Method 7—micro-matrix solid-phase dispersion (μ-MSPD)—was performed by preparing the sample-sorbent column in a glass Pasteur pipette. μ-MSPD allows extraction and purification to be performed in one step and is easy and cheap to perform. This extraction method (14 UV filters) evaluated by AGREEprep received a high score of 0.36. However, this is not the highest result obtained for microextraction methods. This is due to the use of the largest volume of solvents (10 mL of acetonitrile), which lowers the score of criterion 2. Criterion 10 (*safe procedures for the operator*) was assessed more favorably than the other evaluated procedures. Only with this method, no energy is used for sample preparation. Therefore, criterion 8 scores 1 point and is green. However, the AGREE rating of this procedure in criterion 9 regarding energy consumption is red. This is related to the use of an energy-intensive MS/MS detector for the analysis. In addition, lowering the total score results in not using bio-based reagents (criterion 10). However, the overall AGREE score of Method 7 is high, at 0.48.

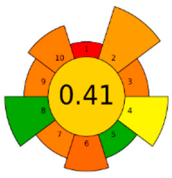
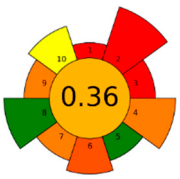
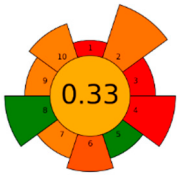
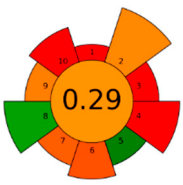
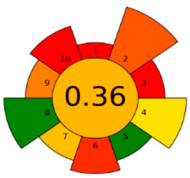
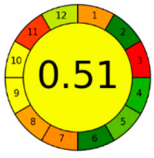
In the case of Method 8—magnetic nanoparticle-based dispersive solid-phase microextraction (MNP-DSPME)—magnetic nanoparticles are used for the separation of the sorbent from the sample solution and eluting the analyte. Due to this, energy consumption decreases and the time of sample preparation is shortened by replacing the centrifugation step with a magnetic field [17]. This results in the method achieving a high greenness score of 0.33 for AGREEprep and the highest score of 0.52 for AGREE. The AGREEprep score was lowered mainly because the authors of the paper tested only 3 UV filters. In addition, criterion 4 (red color) significantly lowers the assessment of this method, mainly due to the waste of 500 mL of water used to dilute the sample, which is contaminated with acetonitrile. On the other hand, two criteria have a green character, low sample consumption (0.02 g, criterion 5), and low energy consumption (criterion 8). The high rating of AGREE (0.52) was influenced by four criteria that received a green color: 2—sample size, 4—number of steps, 6—no derivatization agents, and 12—operator's safety. The miniaturization of the method (criterion 5), low energy consumption (criterion 9), and the use of MeOH as an eluent in HPLC (criterion 10) also contributed to the high overall AGREE scoring.

Ultrasound–vortex-assisted dispersive liquid–liquid microextraction (US–VA–DLL ME)—Method 9—is a simple and cheap sample preparation procedure. The mass transfer

rate of the extraction process was increased by a combination of vortexing (4 min) and ultrasonication (3 min). US-VA-DLLME uses small amounts of extraction solvents (1.57 mL) and a small sample volume (12.5 mg). In addition, bio-derived solvents (anisole) and MeOH were used as extractants and dispersants, respectively. Despite the advantages presented above, this method received a relatively low AGREEprep rating of 0.29. This assessment was mainly influenced by criterion 4 (minimize waste; red color), in which the water used to dissolve the sample was classified as waste (51.6 mL) because it was in contact with the solvent (acetone). In addition, the red character was given to criterion 10 (*use safe reagent*), because the reagents used had four different pictograms. The AGREE score of 0.47 was influenced by the same factors described above. In addition, due to the use of eluents in HPLC analysis (7.5 mL of acetonitrile), the number of reagents used (criterion 11) and waste generated (criterion 7) increased.

Compared to the other procedures evaluated (Table 3), method 10—dynamic hollow fiber liquid-phase microextraction (HF-LPME) with HPLC-UV was well evaluated with scores of 0.36 and 0.51 for AGREEprep and AGREE, respectively. This method owes such good results to the use of a UV detector (low energy consumption) for analysis and the generation of a small amount of waste. HF-LPME was adopted as a semi-automated system, which contributed well to criterion 7 in the AGREEprep evaluation. However, the assessment is negatively affected by the use of 3.01 mL of solvents (hexane, acetone, and toluene) for sample preparation, which in total have 5 different pictograms, which means that criterion 10 in AGREEprep has a low scoring (red color). Also in the AGREE assessment, this has a negative impact on criterion 12, because the use poses a risk to the operator and the environment (mainly due to the use of hexane). In addition, 16 mL of methanol is used to perform the HPLC analysis, which increases the volume of solvents used and the low score of criterion 11 (amount of toxic reagents).

Table 3. Greenness results of methods 6–10 evaluated by AGREEprep and AGREE metrics.

Metric	Method 6 [19] MEPS-GC/MS	Method 7 [21] µ-MSPD-GC-MS/MS	Method 8 [22] DSPME-HPLC-DAD	Method 9 [23] UV-DLLME-HPLC-DAD	Method 10 [24] Dynamic HF-LPME-HPLC-UV
AGREEprep					
AGREE					

3.2. Greenness Assessment Summary

As expected, the microextraction methods (Table 3) showed a more environmentally friendly effect. When scored with AGREEprep, they achieved higher greenness scores, ranging from 0.29 to 0.41 for US-VA-DLLME and MEPS, respectively. On the other hand, for classical extraction methods, the evaluation results were 0.19–0.33 (Table 2). Similar results were obtained when assessing the greenness of entire procedures by AGREE metric. For procedures using microextraction methods, their score is high and amounts to 0.47–0.52 for US-VA-DLLME-HPLC/DAD and DSPME-HPLC/DAD, respectively. Lower results (0.38–0.48) were obtained by procedures using classical extraction methods. The AGREE score is largely affected by the type of chromatographic analysis performed. Due to the solvents used in liquid chromatography, this method of analysis adversely affects the assessment of the greenness of the entire procedure. In addition, in many cases, the use

of MS and MS/MS detectors (more energy-intensive) significantly lowered the overall assessment of greenness (equally for GC and HPLC).

It should also be noted that both AGREEprep and AGREE assessment methods are not always compatible in assessing the greenness of procedures. This is most evident in the case of the DSPME-HPLC/DAD method, where the sample preparation score is low at 0.33, while AGREE rated this procedure at most at 0.52. Similarly for the UV-DLLME-HPLC/DAD method, for which the AGREEprep score was 0.29, and for AGREE, it was 0.47. This proves that it is justified to evaluate procedures by analysts using two assessment tools. If it is necessary to choose an analytical method, only such an assessment will show the positive and negative sides of each procedure and the possibility of improving its greenness.

Selected procedures presented in the literature were not prepared by their authors in terms of greenness assessment. The aim of this work was mainly to obtain positive validation parameters. Therefore, in each of these procedures, criteria can be indicated, the greenness of which can be easily improved. The AGREE assessment is influenced by the number of analytes determined (sample throughput): in this criterion, the assessed methods showed a large discrepancy (from 1 to 17 analytes). This is a criterion that can be corrected for each chromatography system. Another example of improving the greenness of the SPE method can be obtained by extracting multiple samples simultaneously. Similarly, in Method 5—PLE—in the case of using a multi-station ASE extractor, it is possible to improve such criteria as sample throughput, and energy consumption. In the case of the MEPS method, it can be performed in an automatic system, which will improve criterion automatization. In other methods, the amounts of solvents used (amount of toxic reagents) and execution times (sample throughput, energy consumption) in such steps as dissolving cosmetics (mixing and ultrasonication) can be shortened. Moreover, the great impact on the assessment of the method has the type of detector used, if it is possible less energy-intensive detectors, such as FID, ECD, UV-Vis, and DAD, can be used for routine tests.

4. Conclusions

The procedures for determining UV filters in cosmetic samples, due to the complexity of the matrix, are a multi-stage process that consumes large amounts of toxic solvents, time, and energy. The paper presents a comparison of the greenness assessment of 10 analytical procedures that use gas and liquid chromatography techniques. Two recent greenness assessment tools, AGREE and AGREEprep, were used to evaluate these methods. Microextraction methods obtained higher greenness scores than classical methods. For the AGREEprep assessment, the highest score (0.41) was given to the MEPS (Method 6) and solvent extraction with derivatization (Method 3). However, the assessment of the entire procedure (AGREE) indicated that the DSPME-HPLC/DAD (Method 8) and dynamic HF-LPME-HPLC/UV (Method 10) methods were the most "green", with results of 0.52 and 0.51, respectively. Both tools show the advantages and disadvantages of each step of the analytical procedure. The performed assessment of the methods shows that the selection of microextraction methods for sample preparation and low-energetic detectors for analysis is recommended to obtain the eco-friendly analytical method. The assessment tools can be a convenient tool for assessing whether the method is environmentally friendly, but the accuracy and precision of the chosen method should also be taken into account.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/analytica4040032/s1, Descriptions of Methods 1–10; Table S1: Results for the Methods 1–10 have been obtained from Analytical Greenness reports; Table S2: Results for the Methods 1–10 have been obtained from AGREEprep reports.

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Supplementary Materials

Comparison of the greenness assessment of chromatographic methods used for analysis of UV filters in cosmetic samples

Grażyna Wejnerowska and Izabela Narloch *

Descriptions of Methods 1-10:

Method 1 - solvent extraction followed by ultrasonication (in case of determination of the fat-soluble UV filters): 0.1 g cosmetic samples were dissolved with 25 mL of EtOH, followed by 10 min ultrasonic treatment. Next, 3 mL of the ethanolic sample stock solution was transferred to a 10 mL volumetric flask, and the flask was filled up to the mark with EtOH. Then, the extracted solvent was filtered using a 0.45 μm filter, and HPLC-UV/Vis analysis was performed (run time 40 min, 15 analytes). Additional reagents were used for HPLC analysis (mobile phase): 29.9 mL of EtOH and 10.1 mL 1% formic acid solution containing 20 mmol HP-β-CD.

Method 2 – solvent extraction followed by ultrasonication: 0.1 g cosmetic samples were dissolved with 4 mL of MeOH, followed by 30 min ultrasonic treatment. After the supernatant was collected, ultrasonic extraction was repeated twice with 3 and 2 mL of MeOH, respectively. Extracts were combined and reconstituted in 10 mL of MeOH, filtered through a 0.22 μm filter, and HPLC-MS/MS analysis was performed (run time 16.5 min, 5 analytes). Additional reagents were used for HPLC analysis (mobile phase): 2.5 mL of MeOH, and 2.5 mL of acetonitrile.

Method 3 - extraction with derivatization GC-MS/MS: 100 μL nail samples were dissolved with 700 μL of acetone, 100 μL of *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide and 100 μL of internal standards. The samples were vortexed (3 min) and sonicated (20 min). The samples were again vortexed (3 min) and centrifugated (10 min). The supernatant was filtered through a 0.22 μm filter, and GC-MS/MS analysis was performed (run time 18 min , 1 analyte).

Method 4 – solid phase extraction (SPE): 0.1 g cosmetic samples were dissolved in 1000 mL of H_2O. The solutions were mixed using the magnetic stirrer for 15 min. The cartridges (C18, 1000

mg, 6 mL) were conditioned with 5 mL of ethyl acetate (EA) and 5 mL of dichloromethane (DCM). Next, extraction of 100 mL solutions were performed and the analytes were eluted with 5 mL mixture of EA/DCM (1:1, v/v). The eluates were evaporated to dryness under a gentle stream of N_2 at room temperature. The residues were redissolved in 0.5 mL of EA for GC-MS analysis (run time 20 min, 3 analytes).

Method 5 – accelerated solvent extraction (ASE) with single cell – pressurized liquid extraction (PLE): the mixture of 0.1 g cosmetic samples, 0.1 g of anhydydrate Na_2SO_4, and 0.8 g of Florisil were prepared. A cellulose filter was placed at each end of the PLE cell and the mixture was transferred to the PLE cell. Finally, the dead volume of the cell was filled with Florisil. The 10 mL of acetonitrile was used as solvent, and time of extraction was 11 min. The extracts were then derivatized by adding 0.2 mL of acetic anhydrate and 0.01 mL of pyridine. The solutions were maintained at 100°C for 60 min, and then allowed to cool down before GC-MS/MS analysis (run time 14 min, 16 analytes).

Method 6 – microextraction by packed sorbent (MEPS): 0.3 g cosmetic samples were dissolved in 500 mL of H_2O. The sorbent bed was conditioned by flushing 250 μL of EA/DCM (1:1, v/v) and 250 μL of water. Next, 2 mL of the sample was extracted – eight times × 250 μL. Then, the sorbent was washed with 250 μL of H_2O, and the cartridge was dried by pumping air through it (ten times × 250 μL). The analytes were eluted with 100 μL of EA (two times × 50μL) and GC-MS analysis was performed (run time 20 min, 3 analytes). After elution, the cartridge was washed three times with 250 μL of EA and three times with 250 μL of EA/DCM (1:1, v/v).

Method 7 – micro-matrix solid-phase dispersion (μ-MSPD): 0.1 g cosmetic samples were blended with 0.4 g of the anhydrate Na_2SO_4, and 0.4 g of the corresponding dispersing agent (Florisil or sand) until a homogenous mixture was obtained (5 min). The mixture was then

transferred into a 15 cm glass Pasteur, with glass wool at the bottom, containing 0.1 g of Florisil, and 1 mL of acetonitrile, and analyzed by GC-MS/MS (run time 27 min, 14 analytes).

Method 8 – stearic-acid-modified magnetic dispersive solid-phase microextraction (SA-MDSPME): 20 mg cosmetic samples were dissolved with 1 mL of acetonitrile, vortexed (4 min), and centrifugated (1 min). The solution was diluted 500 times with H_2O. Next, 2 mL of the sample solution was buffered with 1 mL of phosphate buffer solution (pH 2.5). The solutions were transferred into 20 mg of preconditioned SA-MNPs as adsorbent and vortexed (1 min). An external magnetic field was used to collect the analyte-rich SA-MNPs, and the supernatant was discarded. The analyte desorption was performed using 100 μL of acetonitrile as the eluent by vortex (1 min). The collected solution was diluted two times with H_2O, and analyzed by HPLC-DAD (run time 6 min, 3 analytes).

Method 9 – ultrasound-vortex-assisted dispersive liquid-liquid microextraction (US-VA-DLLME): 12.5 mg cosmetic samples were dissolved in 50 mL mixture of AC/H_2O (1:39, v/v), then 140 μL of MeOH (dispersant), and 160 μL of anisole (extractant) were added. The sample was vortexed (4 min) and ultrasonicated (3 min). The resulting cloudy solution was then centrifuged for 1 min. The 165 μL bottom phase was dried at 55°C, and the residue was re-dissolved in 20 μL of 2-vinyl naphthalene. The HPLC-DAD analysis was performed (run time 30 min, 5 analytes). Additional reagents were used for analysis (mobile phase): 7.5 mL of acetonitrile, and 0.033 mL of formic acid.

Method 10 - dynamic hollow fiber liquid-phase microextraction (dynamic HF-LPME-HPLC-UV): 0.01 g cosmetic samples were dissolved in 2 mL n-hexane. The samples were ultrasonicated (5 min) and centrifuged (3 min). The supernatant was filtered through a 0.22 μm filter dried at 60°C by nitrogen, and then sample was diluted to 100 mL with de-ionized water. In the meantime, the hollow fiber segments were ultrasonically cleaned in acetone (15

min) and then dried in the air. A disposable flow control valve line for the visiprepTM-DL as the external tube was installed on the sample injection syringe. Then an aqueous sample of 0.6 mL was loaded into the syringe. The hollow fiber attached to the microsyringe needle was inserted into the visiprep TM-DL external tube, and then 10 μL acceptor phase (toluene) was filled into the hollow fiber. The sample was continuously injected into the extractor by the pump. During the extraction, the analytes in the aqueous sample were largely extracted into the organic solvent by diffusion. The analyze-enriched acceptor phase was directly collected into the microsyringe after the extraction. Finally, 5 μL acceptor phases were used for HPLC analysis. The extraction was performed at ambient temperature (25 °C) for 10 min. The HPLC-UV analysis was performed (run time 25 min, 5 analytes). Additional reagents were used for analysis (mobile phase): a mixture of methanol-pure water (80:20, v/v).

Table S1. Results for the Methods 1-10 have been obtained from Analytical Greenness reports

Crite-rion	Criterion description	Weig-ht	Scores									
			Method 1	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7	Method 8	Method 9	Method 10
1.	Direct analytical techniques should be applied to avoid sample treatment	2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
2.	Minimal sample size and minimal number of samples are goals	2	0.98	0.98	0.98	0.98	0.98	0.82	0.98	1.0	1.0	1.0
3.	If possible, measurements should be performed in situ	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4.	Integration of analytical processes and operations saves energy and reduces the use of reagents	2	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	0.8	0.8
5.	Automated and miniaturized methods should be selected	2	0.0	0.0	0.0	0.0	0.25	0.5	0.5	0.5	0.5	0.75
6.	Derivatization should be avoided	2	1.0	1.0	0.51	1.0	0.37	1.0	1.0	1.0	1.0	1.0
7.	Generation of large volume of analytical waste should be avoided, and proper management of analytical waste should be provided	2	0.12	0.31	0.54	0.34	0.36	0.57	0.37	0.0	0.15	0.27
8.	Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time	2	0.58	0.18	0.0	0.15	0.51	0.3	0.59	0.34	0.34	0.32
9.	The use of energy should be minimized	2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5
10.	Reagents obtained from renewable sources should be preferred	2	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5
11.	Toxic reagents should be eliminated or replaced	2	0.0	0.15	0.51	0.15	0.19	0.48	0.2	0.26	0.0	0.12
12.	Operator's safety should be increased	2	0.8	0.8	0.8	0.8	0.6	0.8	0.8	0.8	0.6	0.6
Sum			0.48	0.43	0.39	0.38	0.38	0.48	0.48	0.52	0.47	0.51

Table S2. Results for the Methods 1-10 have been obtained from AGREEprep reports

Crite-rion	Criterion description	Weig-ht	Scores									
			Method 1	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7	Method 8	Method 9	Method 10
1.	Sample preparation placement	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	Hazardous materials	5	0.0	0.0	0.35	0.0	0.0	0.31	0.0	0.23	0.27	0.17
3.	Sustainability and renewability of materials	2	0.0	0.0	0.0	0.0	0.0	0.25	0.0	0.0	0.0	0.0
4.	Waste	4	0.06	0.23	0.44	0.2	0.23	0.49	0.24	0.0	0.0	0.44
5.	Size economy of the sample	2	1.0	1.0	1.0	1.0	1.0	0.84	1.0	1.0	1.0	1.0
6.	Sample throughput	3	0.16	0.0	0.04	0.0	0.0	0.21	0.16	0.16	0.16	0.1
7.	Integration and automation	2	0.25	0.25	0.25	0.19	0.5	0.25	0.25	0.25	0.19	0.38
8.	Energy consumption	4	1.0	0.67	0.84	0.69	0.02	0.85	1.0	1.0	0.87	0.98
9.	Post-sample preparation configuration for analysis	2	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
10.	Operator's safety	3	0.5	0.25	0.5	0.25	0.25	0.25	0.5	0.25	0.0	0.0
Sum			0.33	0.26	0.41	0.26	0.19	0.41	0.36	0.33	0.29	0.36

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A Comprehensive Assessment of Sample Preparation Methods for the Determination of UV Filters in Water by Gas Chromatography–Mass Spectrometry: Greenness, Blueness, and Whiteness Quantification Using the AGREEprep, BAGI, and RGB 12 Tools

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Abstract: Sample preparation is a key step in the analytical procedure. This step is a time- and labor-consuming process, and often it is also expensive, with costs being influenced by the consumption of materials and reagents. Additionally, the toxicity of the reagents, waste generation, and energy consumption affect the environment and the safety of the analyst. New trends in sample preparation are focused on the development of miniaturized methods that are consistent with the principles of green sample preparation and contribute to environmental sustainability. The results of a comprehensive assessment of ten methods of preparing water samples for the determination of UV filters using gas chromatography are presented. Three assessment tools were used for this purpose: AGREEprep (the analytical greenness metric for sample preparation), BAGI (the blue applicability grade index), and the RGB 12 algorithm (red–green–blue model). All the differences and similarities between the three aforementioned metrics are discussed in this manuscript. The results of the evaluation of the most frequently used microextraction methods show their ecological friendliness, effectiveness, and practicality. The results of this assessment will allow researchers to identify the strengths and weaknesses of the given methods and select those that meet their requirements.

Keywords: assessment tools; gas chromatography; greenness; microextraction methods; UV filters

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1. Introduction

UV filters are a group of chemicals commonly used in a wide range of cosmetic products to protect the skin from the harmful effects of UV radiation [1,2]. Organic UV filters have a highly lipophilic character, and most of them are classified as water-resistant and therefore tend to accumulate in the fatty tissues of living organisms [3,4]. They are considered emerging contaminants since they easily enter the natural environment, where they accumulate, causing harmful effects on flora and fauna despite being present at the ng/L level. Therefore, developing sensitive and selective analytical methods for their environmental monitoring is of high interest [5]. As exemplified by the Web of Science database, the results for the combination keywords "UV filters" and "environmental water" showed a growing trend in the amount of research (from 2002 to 2023) on the contamination of the waters by UV filters (Figure 1).

Based on the results obtained from the database, an increasing number of applied microextraction techniques compared to conventional techniques can be observed. The most applied classic extraction methods include solid-phase extraction (SPE), liquid–liquid extraction (LLE), fabric phase sorptive extraction (FPSE), Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction (QuEChERS), and magnetic nanoparticles dispersive solid-phase extraction (MNPs-based dSPE). Microextraction techniques include

solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), dispersive solid-phase extraction (dSPE), microextraction by packed sorbent (MEPS), bar adsorptive microextraction (BAμE), stir bar sorptive dispersive microextraction (SBSDME), single-drop microextraction (SDME), in situ suspended aggregate microextraction (iSAME), hollow-fibre liquid-phase microextraction (HFLPME), dispersive liquid–liquid microextraction (DLLME), ultrasounds-assisted dispersive liquid–liquid microextraction (USA-DLLME), vortex-assisted dispersive liquid–liquid microextraction (VA-DLLME), and ultrasounds-assisted emulsification microextraction (USAE-ME). Based on the literature review, it is concluded that the most popular microextraction techniques for determining UV filters are the following: SPME (~24%); DLLME, with various variants (~24%); SBSE (~16%); MSPE (~5%); and others (~16%). For instrumental techniques, the most common choices for the detection and quantification of the compounds studied in water samples remain gas chromatography and liquid chromatography, coupled with mass spectrometry [5–8].

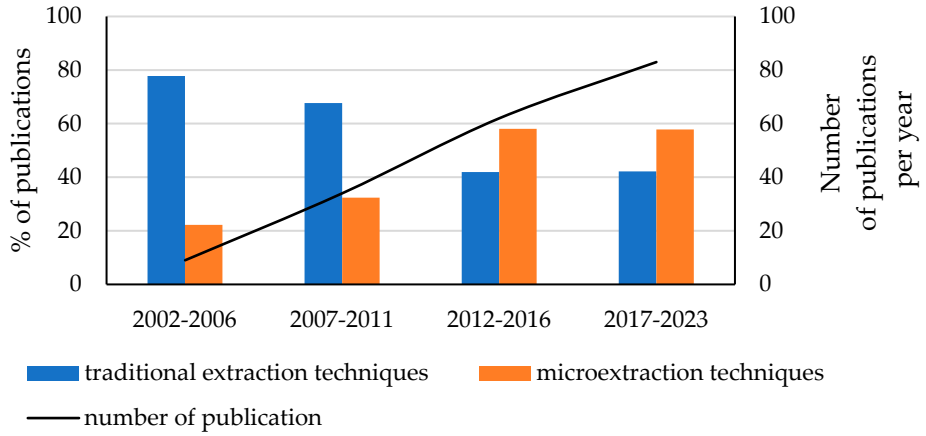


Figure 1. Evolution of the number of publications (%) concerning the determination of UV filters in environmental water samples (2002–2023) by using traditional extraction techniques and microextraction techniques (188 articles on the determination of UV filters in water samples).

Selecting the most appropriate analytical method for determining UV filters in water samples, among many developed methods, is not an easy task. When choosing a method, validation criteria should be taken into account, i.e., accuracy, precision, sensitivity, and selectivity, as well as economic and practical factors, i.e., costs, time, and ease of use. Moreover, special attention is currently being paid to the ecological aspect of the analytical method. According to the principles of Green Analytical Chemistry (GAC), methods should be used that do not pose a threat to human health and the environment.

GAC is an aspect of green analytical chemistry that was introduced in the late 1990s. It is a concept that is based on twelve principles related to the environment, health, and safety [9]. GAC takes into account, among other things, the use of safe solvents/reagents, the generation of toxic waste, and the safety of the analysts. In 2022, López-Lorente et al. [10] proposed ten principles of Green Sample Preparation (GSP) that aim to develop greener analytical procedures. In GSP, the GAC principles have been extended to include the use of solvents/reagents from renewable sources and reusable and/or recyclable materials. Additionally, GSP takes into account sample throughput, miniaturization, and the automation of the method. However, in 2021, Nowak et al. [11] introduced a new concept of sustainable development in analytical chemistry, the so-called White Analytical Chemistry (WAC), which is an extension of GAC. The authors of WAC proposed the WAC principles as an alternative to the 12 GAC principles but including not only green aspects (such as the toxicity of reagents, the number and amount of reagents and waste, energy, and other media, as well as other direct impacts). WAC also takes into account criteria such as analytical efficiency (scope of application, limits of quantification and detection, precision, and accuracy) and practical/economic criteria (cost-efficiency, time-efficiency,

requirements, and operational simplicity). The compliance of analytical methods with the GAC, GSP, and WAC principles is a basic requirement in the development of sustainable analytical methods.

Over the last few years, various metric tools have been introduced to assess the environmental performance of analytical methods, including the National Environmental Method Index (NEMI) [12], the Analytical Eco-Scale [13], the Green Analytical Procedure Index (GAPI) [14], the Analytical Greenness Calculator (AGREE) [15], the RGB 12 algorithm [11], the Analytical Method Greenness Score (AMGS) [16], the Blue Applicability Grade Index (BAGI) [17], the Complementary Green Analytical Procedure Index (Complex-GAPI) [18], and the Analytical Greenness Metric for Sample Preparation (AGREEprep) [19]. All of these tools graphically and/or numerically reflect the compliance of a given analytical method with the GAC principles.

The main aim of this work was to assess the ecological and practical aspects of the water sample preparation step for the determination of UV filters by GC-MS using the AGREEprep, BAGI, and RGB 12 tools. These are the newest tools that are most often chosen for the evaluation of analytical procedures due to their versatility, usefulness, and simplicity of use.

Using these three metrics simultaneously will provide comprehensive information about the strengths and weaknesses of the analytical procedures used. At the same time, the presented correlations between the used metrics can be a guide for the analyst when deciding on the selection of a metric tool.

The assessment of analytical methods is necessary to understand their impact on the environment and can be helpful for analytical chemists when choosing a method for determining cosmetic ingredients and other compounds in water samples.

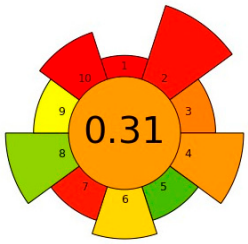
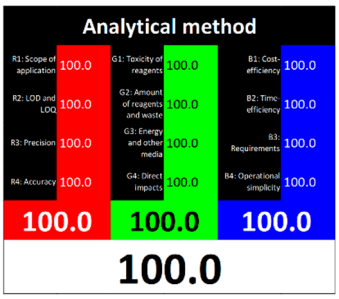
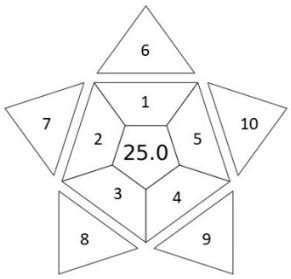
2. Materials and Methods

2.1. AGREEprep

AGREEprep is a new analytical "greenness" metric that was published by Wojnowski et al. [19] in 2022. The free version of the software can be obtained from https://mostwiedzy.pl/AGREE (accessed on 3 May 2024) [20]. AGREEprep is based on ten steps of assessment that correspond to the ten principles of GSP. In order to assess the "greenness" of an analytical method, AGREEprep is based on ten individual steps which are presented in Table 1. Each criterion is scored from 0 to 1, with the extremes representing the worst and best performance, respectively. Moreover, each criterion has a default weight taken into account in the overall score, but the assessors can change this value at their discretion if there are valid reasons to do so [20]. However, in our work, we did not change the value of the criteria because we considered the default weights assigned to the criteria to be correct.

The result of the AGREEprep assessment is a colorful round pictogram that maps the degree of compliance of evaluated criteria within the rules of GAC. The color of the circle inside the pictogram and the overall score within it indicate the overall environmental performance of the sample preparation in a given analytical method. The overall score can range from 0 to 1, with a score of 0 being the worst result and 1 being the best result, taking into account the scores from all criteria or the lack of a sample-preparation step. On the outer part of the circle, there are ten parts, corresponding to the ten criteria. Each part may have a different length, depending on the weight assigned to a given criterion. However, the color of a given part indicates its score—a highest criterion score is indicated in green, and a lowest criterion score is indicated in red. A result between 0 and 1 is represented by a color gradient between red and green, e.g., yellow and orange in different shades, ac-cording to the value assigned by the AGREEprep calculator.

Table 1. Description of the criteria and graphical presentation of results for AGREEprep, WAC, and BAGI metrics.

AGREEprep	WAC	BAGI
1. Favor in situ sample preparation 2. Use safer solvents and reagents 3. Target sustainable, reusable, and renewable materials 4. Minimize waste 5. Minimize sample, chemical, and material amounts 6. Maximize sample throughput 7. Integrate steps and promote automation 8. Minimize energy consumption 9. Choose the greenest possible post-sample preparation configuration for analysis 10. Ensure safe procedures for the operator	RED R1: Scope of application R2: LOD and LOQ R3: Precision R4: Accuracy GREEN G1: Toxicity of reagents G2: Amount of reagents and waste G3: Energy and other media G4: Direct impacts BLUE B1: Cost-efficiency B2: Time-efficiency B3: Requirements B4: Operational simplicity	1. The type of analysis 2. The number of analytes that are simultaneously determined 3. The analytical technique and required analytical instrumentation 4. The number of samples that can be simultaneously treated 5. Sample preparation 6. The number of samples that can be analyzed per hour 7. The type of reagents and materials used in the analytical method 8. The requirement for preconcentration 9. The automation degree 10. The amount of sample
		

2.2. WAC

White analytical chemistry (WAC) is a concept of sustainable development in analytical chemistry, which is an extension of green analytical chemistry. WAC was designed and developed by Nowak et al. [11] in 2021, and it is a concept that encourages the harmony and integration of analytical, ecological, and practical characteristics, while aiming for the sustainability of analytical methods. In the WAC concept, the RGB (red, green, blue) model [21] is used to evaluate the analytical method. Just as the color white is created by mixing red, green, and blue light, the analytical method becomes white, and thus complete, when it achieves each primary color.

To evaluate the methods using the RGB 12 algorithm, which is the second version of the RGB model adapted to the 12 criteria of WAC, the available Excel template spreadsheet is used (access in Supplementary data in Nowak et al.'s work [11]), where specially prepared tables of red, green, and blue colors can be found. The template was designed to be able to evaluate and compare 10 methods simultaneously. The tables should be completed by assigning each criterion a point value ranging from 0 to 100. A value of 0 means the worst result, and 100 means that the method is well suited to the planned application. It is also possible to award more than 100 points for outstanding criteria in the evaluation of an analytical method. After completing the form, the assessment results are automatically calculated and presented in tabular form. The compliance of the method with a given WAC criterion is presented both numerically and visually by saturating a given value with color (a criterion value of 0 corresponds to black; 100 or more points correspond to full-color saturation). The value of arithmetic means for the red, green, and blue criteria are expressed individually as R (%), G (%), and B (%), while the overall result (whiteness—%) is given in the table and figure (Table 1).

2.3. BAGI

The blue applicability grade index (BAGI) is a new analytical "blueness" metric tool for evaluating the practicality of an analytical method, which was published by Manousi et al. [17] in 2023. The free version of the software can be obtained from https://mostwiedzy.pl/pl/justyna-plotka-wasylka,647762-1/BAGI (accessed on 3 February 2024). The blue color in the BAGI metric is inspired by the RGB model, and it may be considered complementary to the existing green metrics tools. In order to assess the applicability of an analytical method, BAGI takes into account the criteria shown in Table 1.

The overall result of assessing the method using BAGI is an asteroid pictogram with a number in the center. The hue of the scale of the pictogram reflects the compliance of the method with the designated criteria. There are four colors in the BAGI: dark blue for high compliance, blue for medium compliance, light blue for low compliance, and white for no compliance. The number in the center of the pictogram indicates the overall score for the analytical method, which is a number ranging from 25 to 100. A point value of 100 is assigned to a method with excellent performance, and a value of 25 indicates the worst performance of the method in terms of applicability. A method whose BAGI score is at least 60 points is considered practical. In the pictogram, criteria 1–5, located in its inner part, correspond to the stage of analytical determination or sample preparation stage. However, criteria 6–10, placed in the outer part, correspond to both mentioned stages. The result field takes on a shade that is the average shade of all criteria taken into account in BAGI.

3. Results and Discussion

3.1. Greenness, Blueness, and Whiteness Evaluation

The paper presents an assessment of the environmental impact and analytical suitability of ten methods of preparing water samples for the determination of UV filters described in the literature. One of the methods, solid phase extraction (SPE), is a classic extraction method, while the other nine are commonly used microextraction techniques. Table 2 presents assessed analytical methods and their literature sources. A brief description of the assessed sample preparation methods for analysis is presented in Supporting Information (Table S1).

Table 2. Results from the evaluation of methods for preparing water samples for the analysis of UV filters obtained using the AGREEprep, BAGI, and WAC metrics.

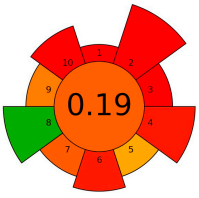
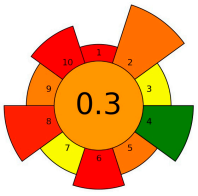
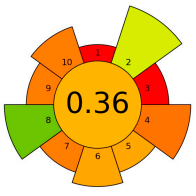
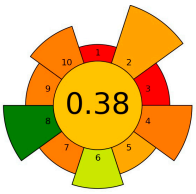
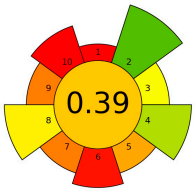
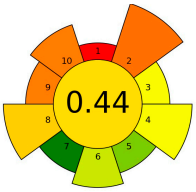
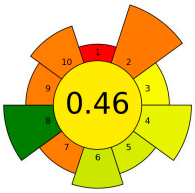
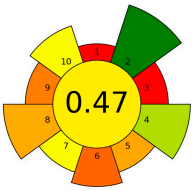
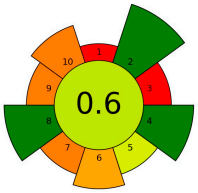
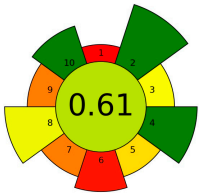
Method	AGREEprep	WAC [%] *	BAGI
SPE—solid phase extraction [22]		R 91.3 G 75.0 B 66.3 W 77.5	
SBSE—stir bar sorptive extraction [23]		R 83.8 G 81.7 B 51.5 W 72.3	
USAEME—ultrasound-assisted emulsification microextraction [24]		R 92.5 G 86.7 B 92.9 W 90.7	

Table 2. *Cont.*

Method	AGREEprep	WAC [%] *	BAGI
DLLME—dispersive liquid-liquid microextraction [25]		R 90.0 G 90.8 B 100.4 W 93.8	
SPME (derivatization)—solid-phase microextraction (with derivatization) [26]		R 95.0 G 96.3 B 67.1 W 86.1	
MEPS (automated)—microextraction by packed sorbent [27]		R 87.5 G 89.6 B 87.9 W 88.3	
MEPS—microextraction by packed sorbent [28]		R 91.3 G 98.8 B 97.1 W 95.7	
DmSPE—dispersive micro solid-phase extraction [29]		R 97.5 G 99.6 B 80.2 W 92.4	
SDME—single-drop microextraction (named by authors of article liquid-phase microextraction [30])		R 95.0 G 101.3 B 103.8 W 100.0	
SPME—solid-phase microextraction [31]		R 93.8 G 106.7 B 72.1 W 90.8	

* R—red principles (analytical performance); G—green principles (green chemistry); B—blue principles (practical side); W—whiteness.

All selected sample preparation procedures use gas chromatography and mass detection (GC-MS) for analysis. This made it possible to evaluate only the sample preparation step without taking into account the time, costs, and energy needed to perform the chromatographic analysis. In the case of methods using thermal desorption of analytes from the sorbent in the chromatograph dispenser, i.e., SBSE and SPME, the desorption step was included in the total assessment (time and energy consumption). It was also assumed that one sample was analyzed, and the time and costs incurred for its optimization were not taken into account when assessing the method. Using the example of determining UV filters in water samples, the environmental friendliness and functionality of commonly used microextraction methods were assessed using three tools, i.e., AGREEprep, BAGI, and RGB 12. AGREEprep assesses in detail the environmental impact of the use of a given analytical procedure, BAGI assesses its usefulness, while RGB 12 covers its comprehensive assessment ecological performance, analytical performance, and its economic and practical aspects.

3.1.1. AGREEprep Assessment

The use of AGREEprep allows the critical evaluation of each step of sample preparation from the GAC point of view. The results of the assessment performed using AGREEprep are presented in Table 2. As expected, the lowest score (0.19) was obtained by the SPE method, in which only one of the principles, energy use, was green (criterion 8). Classic extraction methods, including SPE, are characterized by high consumption of toxic solvents (criterion 2 uses safer solvents, and criterion 10 is safe for the operator), generate large amounts of waste (criterion 4), and are time-consuming (criterion 6). The SPE method, among the classical methods, is the most frequently used method for analyzing UV filters, which is the reason for presenting it in this work to compare it with microextraction methods.

However, the lowest AGREEprep score among the presented microextraction methods was given to the SBSE method, whose score was 0.3. Such a low assessment of the greenness of this method is due to the long time (criterion 6) of extraction (180 min) and desorption (15 min), as well as the energy (criterion 8) consumed during sample mixing, especially during the desorption step.

The USAEME method received a low AGREEprep score of 0.36. Only 100 µL of chloroform was used for extraction, which has a high score effect on the assessment of criterion 2. In this procedure, a large amount of sample was used (10 mL; criterion 5), to which 2 g of NaCl was added, together with the solvent. Due to the solvent contamination of the aqueous solution, all of the solution was treated as waste, which significantly lowered the score for criterion 4.

Sample preparation using the DLLME method takes very little time (~5 min), which results in a favorable result for criterion 6 (sample throughput). In this method, the sample is subjected to a short centrifugation (3 min), thanks to which the energy consumption is low, which is why criterion 8 has a green rating. However, the use of two hazardous solvents (1 mL acetone and 60 µL chlorobenzene) negatively affects criteria 2 and 10 (use safer solvents and safe for the operator), and it also results in a low AGREEprep score of 0.38.

The UV filters contain phenolic hydroxyl groups, which cause the low sensitivity of the GC analysis. The use of derivatization increases sensitivity and improves separation and shape peaks. Therefore, derivatization is often used in their analysis. Derivatization (on-fiber silylation) was used in the next assessed method—SPME. However, its use has a negative impact on the assessment of the ecological effectiveness of the method. The SPME with the derivatization method received a score of 0.39 in the AGREEprep evaluation. The reagents used for sample acidification and derivatization (MSTFA), and the time needed to perform derivatization, extraction, and desorption (~45 min) reduce the scores for criteria 6 and 10 (sample throughput and safety for the operator). Additionally, the energy consumed for mixing, sample heating, and thermal desorption reduces the score for criterion 8. For comparison, the SPME procedure (without derivatization) obtained the highest result of

0.61 among the assessed methods. A much smaller impact on the reduction of the greenness rating due to derivatization was demonstrated for other methods, i.e., DLLME (ultrasound-assisted) [32] and SBSE [33]. The AGREEprep rating for the DLLME method decreased from 0.38 to 0.3, and for SBSE from 0.3 to 0.28. In these methods, unlike SPME, derivatization is carried out simultaneously with extraction, which does not result in increased time and energy consumption.

The example of the MEPS method shows the differences in the AGREEprep assessment for the fully automated and manual MEPS method, which received scores of 0.44 and 0.46, respectively. Both methods have one green criterion: the manual MEPS scores green on criterion 8 (energy consumption), while the fully automated method scores green on criterion 7 (integration, automation). However, criterion 8 has a higher weight in the AGREEprep metric, which causes a difference in the evaluation of both methods. The rating of both MEPS techniques is not the highest, influenced by the fact that solvents are used for extraction (low criterion 2), and it additionally reduces the criteria related to waste generation and safety for the operator (criteria 4 and 10).

Dispersive micro- solid-phase extraction (DmSPE) is a solvent-free method, with a positive impact on criterion 2, which received the green status. However, the total score for this procedure is not high, and amounted to 0.47. The derivatization step of analytes reduces the overall evaluation of the method. After extraction, the analytes, together with the sorption bed, are placed in the injection port, where they are derivatized and then thermally desorbed. The use of a derivatization reagent (BSTFA) extends the sample preparation time (criterion 6—sample throughput) and reduces criteria 2 and 10 (use safer reagents and operator safety).

Only two microextraction methods achieved the green status, with scores of 0.6 and 0.61—SDME and DI-SPME, respectively. The SDME method obtained such a high rating compared to the previously discussed methods thanks to the use of a very small amount of solvent (3 µL of toluene) for extraction, which is injected into the injection port after extraction. This affects two green criteria—criterion 2 (use safer solvents) and criterion 4 (minimize waste). The favorable final assessment was also influenced by the use of only 2 mL of sample for analysis (criterion 5) and the consumption of a small amount of energy (criterion 8) due to magnetic stirring for 20 min. Compared to SDME, criterion 8 in the SPME method has a lower score due to the higher energy consumption during the thermal desorption step and the long operating time of the magnetic stirrer during the sorption step (45 min). However, because it is a solvent-free, waste-free method and no derivatization of analytes was used, three criteria obtained a green rating, with a result of 1 (criteria 2, 4, and 10—use safer reagents, minimize waste and operator safety), which resulted in the highest score (0.61) for the SPME method in terms of greenness.

3.1.2. WAC Assessment

The RGB 12 metric, compared to the AGREEprep and BAGI metrics, allows for a deeper analysis of the method in terms of its analytical performance and practical benefits. This metric allowed the authors to assign scores to individual categories according to their discretion (experience and needs). All data of the assessed procedures were compiled in the available Excel spreadsheet, and, taking into account the importance of each criterion, points were assigned to all parameters.

The scores obtained for each principle are presented in Table 2 and Figure 2, and the detailed elements of this assessment are presented in an Excel spreadsheet (Supporting Information).

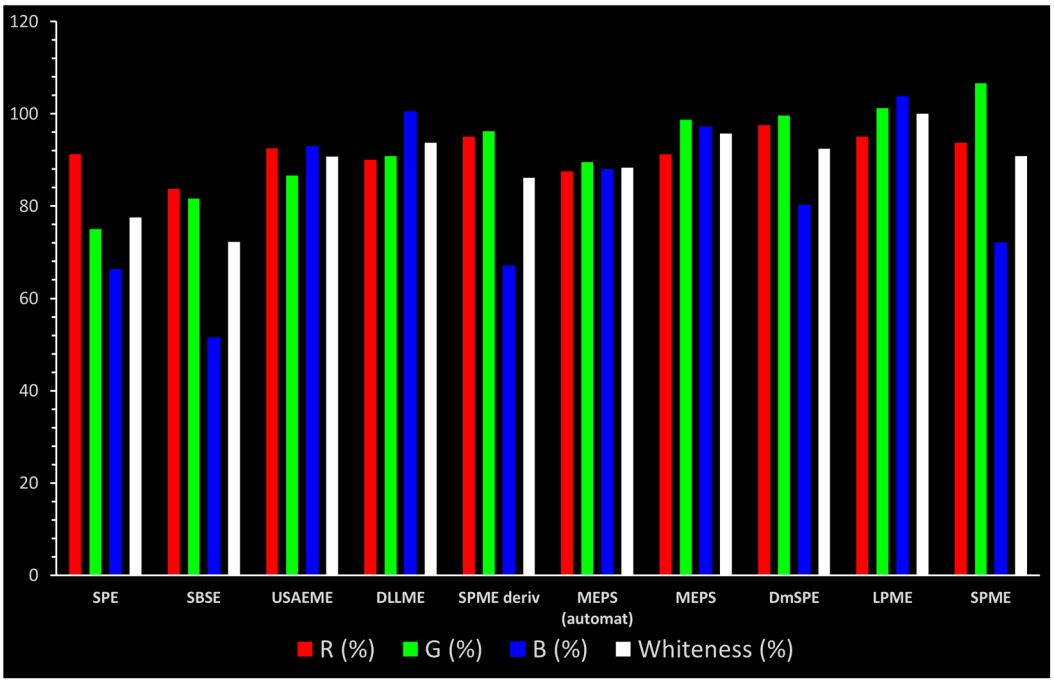


Figure 2. Comparison of the main assessment outcomes from the RGB 12 tool. Values above 100 indicate additional capabilities exceeding current requirements.

RED Principles Rating (Analytical Performance)

The red category evaluates the method in terms of its applicability to its intended purpose. Precision, accuracy, limit of detection, and scope of application are assessed in this category. When determining the UV filters in the environmental samples, this category is of great importance due to the presence of these compounds at very low concentration levels and the wide range of UV filters used.

High redness results of 95% were obtained by the SPME (with derivatization) and SDME methods, while the DmSPE method was rated highest in this category, with a score of 97.5%. Of the assessed parameter components (R2), the lowest LOD (0.5–10 ng/L) was achieved by the SPME (on-fiber silylation) and DmSPE methods, earning them 100 points. These methods also showed high precision and accuracy, which contributed to the high final results in this category.

However, the LODs of the remaining methods were at a similar concentration level. The last points in this category were awarded to the SPE (70%) and SPME (75%) methods, for which the LOD was ~1–8 µg/L. However, it should be taken into account that the LOD was determined in laboratories using MS detectors operating at various parameters.

Green Principles Rating (green chemistry). The authors of this work entered the data of all assessed procedures into the Excel spreadsheet for the following categories: G1: toxicity of reagents, G2: amount of reagents and waste, G3: consumption of energy, and G4: direct impacts (safety). Based on these data, they assigned points from 0 to 120 to individual categories. It was decided to award 120 points to categories that showed 0 (reagents, waste, energy).

The greenest methods according to WAC are SPME, with a score of 106.7%, and SDME, with a score of 101.3%. The SPME method was awarded 120 points for the G1, G2, and G4 criteria. This is because only in this procedure no solvents and reagents were used. However, the SDME procedure requires the use of only 2 µL of solvent, which also contributes to the high rating of this method. Neither method generates waste and both are safe for the operator. The greenness rating of the remaining microextraction methods ranged from 81.7 to 99.6. These differences mainly resulted from the amount of energy used. As expected, SPE received the lowest greenness score (75), which is mainly due to the

large amount of reagents used (~40 mL) and waste generated (30 mL). These parameters mean that for the G2 principle, SPE received only 20 points.

BLUE Principles Rating (Practical Side)

The assessment of practical and economic aspects includes the following categories: B1: cost-efficiency, B2: time-efficiency, B3: requirements, and B4: operational simplicity. In the blue principles, it was decided to distinguish procedures by awarding them 120 points for zero financial contribution to equipment and reagents in category B1, for methods whose sample preparation time is lower than 5 min in category B2, and for methods using less than 1 mL of sample in category B3.

Two of the assessed procedures, SDME and DLLME, received over 100 points: 103.8 and 100.4, respectively. The main advantages of these methods are their low costs and speed of implementation (B1 and B2). The MEPS (manually) and USAEME methods also have high scores, of over 90 points. However, the remaining methods (SBSE, SPME, SPE), due to the costs of purchasing accessories, automatic attachments (e.g., thermal desorption for SBSE), and derivatization reagents, received a low rating for category B1. Additionally, the total rating of these methods was lowered by the B3 category, which is influenced by the requirements for "advanced instruments and greater operator skills and experience".

Whiteness

Whiteness is a summary assessment of the three components (red, green, and blue) and shows the overall usefulness of the procedure. This assessment tool enables the analyst to select a procedure that will meet the given expectations. The assessment results, together with knowledge of the matrix composition, amounts, properties, and expected concentrations of analytes, laboratory equipment, economic opportunities, and analyst skills will help to select the most appropriate procedure from among the highest-rated procedures.

The highest whiteness rating (Table 2, Figure 2) of 100% was awarded to SDME, with only the red principle rated at less than 100 points. Assuming that the authors of this paper consider a result of more than 90% to indicate the whiteness of the method, six out of nine microextraction methods achieved satisfactory results. However, the lowest whiteness ratings were received by SBSE (72.3%) and SPE (77.5%), for which the blue principles had the greatest impact on the assessment.

3.1.3. BAGI Assessment

BAGI evaluates ten main attributes of an analytical procedure in terms of practicality. The results of the assessment performed using BAGI are presented in Table 2. Six of the assessed methods obtained a high score, higher than 60, indicating their practicality. According to this assessment, the highest score was 70 points, obtained by the DLLME and MEPS (automatic) methods, then MEPS (manually), USAEME, and SDME—65 points—and DmSPE—62.5 points.

The remaining methods, i.e., SPE, SPME, and SBSE, received scores in the range of 50–60 points. This assessment is influenced, among other things, by parameter 7 (reagents and materials), which gives a low score for the need to purchase "commercially available reagents and materials" such as SPE cartridges, SPME fibers, SBSE twisters, and derivatization reagents. Additionally, the SBSE and MEPS (fully automated) methods received 0 points for parameter 3 (analytical technique) for "instrumentation that is not commonly available in most labs".

3.2. AGREEprep vs. GREEN Principle of WAC Assessment

It was verified whether the greenness assessment of the methods performed using the AGREEprep tool was consistent with the green principles of the WAC assessment. The methods assessed are presented in Table 2 in ascending order of score (from the AGREEprep assessment), and this order was in most cases confirmed after the assessment of the green principles of WAC. The only discrepancy was observed in the greenness

assessment for the MEPS (fully automated) method, which obtained a higher result in the AGREEprep assessment. This higher score was related to criterion 7, where the automated method receives additional points in the AGREEprep assessment. However, in the RGB 12 algorithm, the automation of methods is assessed in the principle blue: 4 (operational simplicity). Despite this, a high degree of convergence was observed in the greenness assessments of both metrics used to evaluate the ten methods for preparing water samples for the analysis of UV filters using the GC-MS technique. This convergence is shown in Figure 3, which shows that the correlation of both assessments is high and amounts to 0.877.

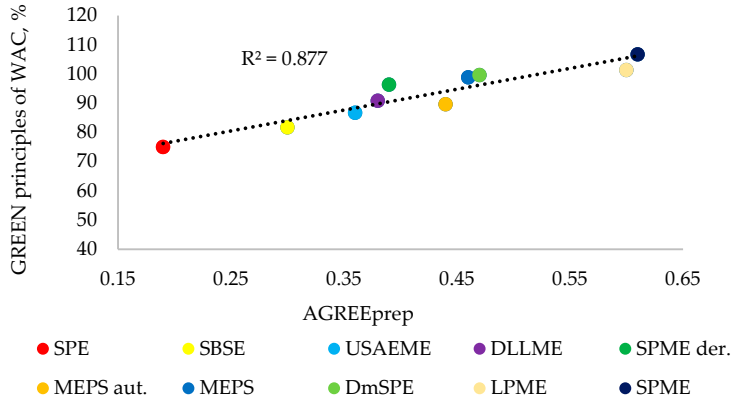


Figure 3. Linear correlation between the AGREEprep and GREEN principles of WAC.

3.3. BAGI vs. BLUE Principle of WAC Assessment

The BAGI tool is used to assess the suitability of an analytical method. However, the RGB 12 algorithm includes the blue principle (practical side), which is one of the components of the total whiteness assessment of the method. The correlation between the results obtained using the BAGI and the blue principle tools is shown in Figure 4. Despite a moderate correlation (R2 ~ 0.7), there was some agreement between both assessments. The four highest-rated methods for suitability by both tools are DLLME, SDME, MEPS, and USAEME. These methods obtained results for BAGI > 60 points, and for the blue principles > 90%. However, the SPME, SBSE, and SPE methods were rated the lowest by both tools. As can be seen in Figure 4, a greater discrepancy in both assessments can be seen for two methods: SPE and MEPS (fully automated). In the case of the SPE method, the BAGI rating is low (50), which is influenced by principle 8 (preconcentration), which lowers the rating for methods that use additional concentration steps (solvent evaporation). However, in the case of the MEPS (fully automated) method, the blue rating in relation to BAGI is lowered by additional parameters assessed in the blue metric tool, i.e., B1 (total cost) and B4 (portability).

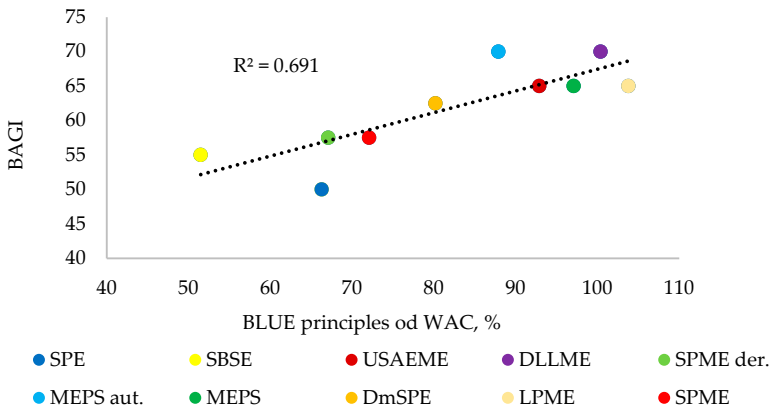


Figure 4. Linear correlation between BAGI and blue principles of WAC.

3.4. Summary of Evaluation of Sample Preparation Methods

The data presented in the Introduction indicate that the most popular microextraction techniques for determining UV filters are SPME (~24%) and DLLME, with various variants (~24%). In the case of the SPME method, this result is very consistent with the AGREEprep evaluation, in which this technique obtained the highest result (0.61). It is a solvent-free and waste-free method, which makes it unrivalled in the "green" category. However, it can be noticed that the best evaluation results in all categories (green, blue, and white) were obtained by the SDME method. However, these highest ratings for the SDME method do not translate into the popularity of using this method in practice (~3%). This is probably mainly related to maintaining a stable solvent microdrop. However, another popular method used by analysts—DLLME—is easy to perform and cheap. Its frequent use coincides with the highest BAGI rating (70 points), indicating its practicality.

These observations confirm the fact that analysts, using their experience, accurately select the most beneficial techniques. These techniques are simple, cheap, fast, and solvent-free or use minimal amounts of solvents. They are also characterized by reliability, repeatability, and sensitivity. Additionally, what is important is that their use does not require the purchase of additional laboratory equipment. However, the evaluation tools used confirm and prove the selection of the most advantageous analytical technique, and they are used to evaluate newly developed procedures.

4. Conclusions

The results of the assessment of sample preparation procedures presented in this work, based on the example of determining UV filters in water samples, demonstrated the usefulness and effectiveness of all metric tools used. The WAC tool was found to evaluate the methods most comprehensively, as expected. The advantage of this tool is that it allows the analyst to independently assign points for individual principles according to individual problems and needs, although this task is time-consuming and difficult to perform. However, the AGREEprep and BAGI tools evaluate the method within a narrower scope (greenness and practicality), and their implementation is relatively simple and quick. As a result of the evaluations, AGREEprep and BAGI were highly consistent with the WAC tool. There is no doubt that the procedure assessment tools used in this work are useful and help the analyst decide which method to choose for use in the laboratory.

As shown by the evaluation carried out using three complementary tools, the SDME, SPME, and DLLME methods were rated the highest. Two methods, i.e., SPME and SDME, obtained the highest greenness results which was confirmed by the green principles of WAC (>100%). However, the best method in the practicality category is DLLME, which received 70 points in the BAGI assessment, and it was also confirmed by the blue principles of WAC (>100%).

The presented assessment results show that the use of expensive materials and devices and conducting additional steps for the procedure, e.g., derivatization, sonication, etc., negatively affect all assessment. The preferred and still-developing methods should be (if possible) simple, cheap, fast, and preferably solvent-free. However, when choosing a method, it is necessary to remember to maintain a balance between greenness, functionality, and usability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14177690/s1, Table S1: A brief description of the analytical methods assessed; Excel spreadsheet: WAC assessment.

Author Contributions: Conceptualization, G.W. and I.N.; formal analysis, G.W.; methodology, G.W. and I.N.; writing—original draft preparation, G.W. and I.N.; writing—review and editing, G.W. and I.N. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Supporting Information

A comprehensive assessment of sample preparation methods for the determination of UV filters in water by gas chromatography: greenness, blueness, and whiteness quantification using the AGREEprep, BAGI and RGB 12 tools

Grażyna Wejnerowska and Izabela Narloch *

Table S1. A brief description of the analytical methods assessed.

Method	Sample preparation procedure	Ref.
SPE - solid phase extraction	The SPE disk was washed with 10 mL of methylene chloride and conditioned with 10 mL of methanol and 10 mL of deionized water. Extraction was carried out using a 10 mL water sample. After extraction, the disk was dried under vacuum for 10 min. Next, analytes were desorbed with 5 mL of methylene chloride. The obtained extract was evaporated under nitrogen to dryness and was dissolved in 0.1 mL of methanol.	[21]
SBSE - stir bar sorptive extraction	The 20 mL of a water sample (containing 10% methanol; pH 2) was placed in the beaker. The twister was added and extraction was performed by stirring (1000 rpm) for 3 h at room temperature. Then, thermal desorption was followed at 250 °C for 15 min.	[22]
USAEME - ultrasound-assisted emulsification microextraction	The 2 g of NaCl and 100 μL of chloroform (extraction solvent) were added to 10 mL of water sample. Extraction was carried out for 5 minutes in an ultrasonic water bath. The emulsion was then broken by centrifugation (3500 rpm) for 10 min. Chloroform was removed using a syringe and transferred to a vial.	[23]
DLLME - dispersive liquid-liquid microextraction	The 1 mL of acetone (dispersant) containing 60 μL of chlorobenzene (extractant) was introduced into 10 mL of water sample. This solution was shaken by hand for 1 min and centrifuged (3200 rpm) for 3 min. A drop of chlorobenzene was transferred to a vial.	[24]
SPME - solid-phase microextraction (with derivatization)	The SPME fiber was exposed directly to 10 mL of water sample (pH 3, room temperature, magnetic stirring) for 30 min. Next, the fiber was placed in the headspace of a vial containing 20 μL of MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide). On-fiber silylation was performed at 45 °C for 10 min.	[25]
MEPS (automated) – microextraction by packed sorbent	The water sample was processed by a Multi Purpose Sampler. The sorbent bed was conditioned by 2 x 50 μL of methanol and deionized water. The analytes were extracted from sample volumes of 800 μL. The sorbent bed was washed with 50 μL water and elution was 2 x 25 μL of ethyl acetate. After the extraction process, 10 wash cycles, each with 100 μL methanol.	[26]
MEPS – microextraction by packed sorbent	The sorbent bed was conditioned by 250 μL of ethyl acetate-dichloromethane mixture (1:1, v/v) and 250 μL of ultrapure water. Next, 2 mL of the water sample was extracted by taking it from a vial and discarding it to waste (8 x 250 μL). Then, the sorbent was washed with ultrapure water (250 μL). The analytes were eluted with 100 μL of ethyl acetate (2 x 50 μL). Finally, after elution, the cartridge was washed three times with 250 μL of ethyl acetate and three times with 250 μL of ethyl acetate-dichloromethane mixture (1:1, v/v).	[27]
DmSPE - dispersive micro solid-phase extraction	The 1.5 mg of adsorbent was immersed in 10 mL of water sample, shaken for 1 min, and the filtered adsorbent was placed in the GC injection port, where the adsorbent was dried for 3.5 min. 2 μL of BSTFA was added to it and derivatized for 2.5 minutes, heated for 5.7 minutes for thermal desorption.	[28]
LPME - liquid phase microextraction	A drop of 3 μL of toluene was held 5 mm below the surface of the water sample. The extraction was mixed for 15 min (500 rpm). Then, the 2μL of the extract was introduced for GC/MS analysis.	[29]
SPME - solid-phase microextraction	The SPME fiber was introduced into a mixed water sample (5 mL) and extraction was carried out for 45 min at room temperature. Desorption of analytes was performed at 250°C for 2 min.	[30]

Publikacja naukowa [P7]

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Nutshell Materials as a Potential Eco-Friendly Biosorbent for the Effective Extraction of UV Filters and Parabens from Water Samples

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Abstract: UV filters and parabens, as ingredients of cosmetics, are commonly occurring water pollutants. In our work, nutshells were used as biosorbents in the developed analytical procedure for the determination of UV filters and parabens in water samples. The shells obtained from walnuts, hazelnuts, peanuts and pistachios were applied as biosorbents. The proposed analytical method can be used as a powerful alternative to other methods for the analysis of UV filters and parabens in water samples. A method of carrying out the sorption step and its parameters, i.e., the effect of time, pH, and salt addition, was developed. A method for the desorption of analytes was also developed, in which the type and volume of solvent, and the desorption time, were established. The recoveries were in the range of 59–117% for benzophenones and lower recoveries from 14 to 75% for parabens. The results showed that nutshells can be used as low-cost, efficient and eco-friendly biosorbents for the determination of parabens and UV filters in water samples. These materials can be used as a 'greener' replacement for the commercially available adsorbents for the extraction of cosmetic ingredients from the environment.

Keywords: benzophenones; biosorbent; emerging contaminants; extraction method; nutshells; parabens; UV filters

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1. Introduction

Due to the fact that UV filters and paraben preservatives are widely used components in many personal care products, they are commonly found in the environment. These substances are an increasingly serious environmental problem, due to their increasing detection in waters and marine biota (e.g., in fish, mollusks, and corals). Depending on the season, location, public access and sampling conditions, the detectability of UV filters and parabens in water ranges from ng/L to mg/L [1]. Growing concerns about the possible harmful effects (e.g., endocrine-disrupting, carcinogenic, neurotoxic, and bioaccumultive) of environmental pollutants have led to an increasing amount of research to determine their ecological and physiological effects [2,3]. Due to the possibility of continuously exposing humans to these chemicals, it is important to monitor and strictly control the water environment in terms of the content of UV filters and parabens in it.

For this purpose, extraction techniques play an important role in sample preparation for the determination of UV filters and parabens in environmental water samples. According to the literature, the most commonly used extraction techniques are liquid–liquid extraction (LLE) and solid-phase extraction (SPE). However, these techniques, due to the consumption of large volumes of samples and organic solvents, as well as high time consumption, have been replaced by microextraction techniques. These techniques include solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), dispersive solid-phase extraction (dSPE), microextraction by packed sorbent (MEPS), bar adsorptive

microextraction (BAμE), and dispersive liquid–liquid microextraction (DLLME) [4–6]. They are characterized by high efficiency, speed, and low costs, as well as the possibility of extensive modification by introducing new materials and solvents.

In recent years, analytical chemistry has been striving to develop analytical procedures based on the principles of Green Analytical Chemistry (GAC) and its development White Analytical Chemistry (WAC). Therefore, both economically and environmentally, attention is paid to the reduction, reuse and recycling of materials used in analytical methods that do not negatively affect analytical performance [7]. For this reason, biosorbents are promising materials in extraction techniques due to their high extraction capacity, non-toxicity, low cost, biodegradability and environmental friendliness [8,9]. Biosorbents are divided into three main groups: microorganisms (fungi, bacteria and algae), chitin/chitosan and lignocellulose. Among the groups mentioned, the most frequently used biosorbent is lignocellulose, which is isolated from plant tissues or obtained from unrefined plant products, such as tree barks or corks [10]. One example of biosorbents consisting of lignocellulose are nutshells. The literature contains studies using these sorbents to remove metals [11–14], pesticides [15,16], dyes [17,18], and pharmaceuticals [19–21]. However, no studies were found regarding the use of nutshells as sorbents used for the determination of personal care products, i.e., parabens and sunscreen filters in water samples.

The aim of this study was the development of an analytical methodology for determining the amounts of UV filters (benzophenones) and parabens in water matrices. For this purpose, for the first time, the walnut, hazelnut, peanut and pistachio shells were applied as sorbents for the analytes. The proposed method is eco-friendly, low-cost, and fast. The optimization, validation, and application of the proposed analytical method to water matrices are fully discussed.

2. Materials and Methods

2.1. Materials and Reagents

Analytical standards of methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), and butyl paraben (BP) were purchased from Sigma-Aldrich (Darmstadt, Germany), while benzophenone (BPZ), benzophenone-1 (BP1), benzophenone-3 (BP3), benzophenone-8 (BP8) and decane, used as internal standard (IS), were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The structures and relevant physicochemical properties of analytes are exhibited in Table 1. These standards were used to prepare a 1 mg/mL stock solution in methanol (MeOH). The stock solution was used to prepare a working solution with a concentration of 0.5 mg/L of each analyte in ultrapure water. The hydrochloric acid (36%), which was used for pH adjustment, and the salting-out effect were evaluated with the addition of sodium chloride (NaCl) obtained from Chempur (Piekary Śląskie, Poland). For re-dissolving analytes, EA/ACN mixture (1:1, *v:v*) with IS at a concentration of 10 μL/L was used. Ethyl acetate (EA), acetonitrile (ACN) and MeOH were supplied from Merck (Darmstadt, Germany) and were analytical grade.

Table 1. The physicochemical properties and structure of the studied compounds.

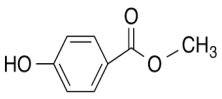
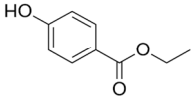
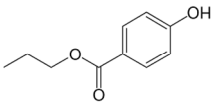
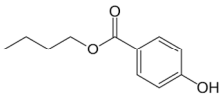
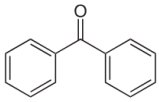
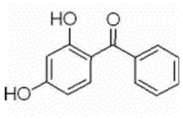
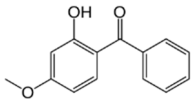
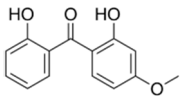
Compound	Abbreviation	Formula	CAS Number	Chemical Structure	Molecular Weight (g/mol)	Log $K_{o/w}$	pK_a
Methylparaben	MP	$C_8H_8O_3$	99-76-3		152.15	1.96	8.17
Ethylparaben	EP	$C_9H_{10}O_3$	120-47-8		166.17	2.47	8.22

Table 1. *Cont.*

Compound	Abbreviation	Formula	CAS Number	Chemical Structure	Molecular Weight (g/mol)	Log $K_{o/w}$	pK_a
Propylparaben	PP	$C_{10}H_{12}O_3$	94-13-3		180.21	3.04	8.35
Butylparaben	BP	$C_{11}H_{14}O_3$	94-26-8		194.23	3.57	8.37
Benzophenone	BPZ	$C_{13}H_{10}O$	119-61-9		182.22	3.18	7.5
Benzophenone-1	BP1	$C_{13}H_{10}O_3$	131-56-6		214.22	3.15	7.53
Benzophenone-3	BP3	$C_{14}H_{12}O_3$	131-57-7		228.24	3.79	7.56
Benzophenone-8	BP8	$C_{14}H_{12}O_4$	131-53-3		244.24	3.82	6.99

Data were obtained from: https://pubchem.ncbi.nlm.nih.gov (accessed on 31 July 2024).

The water sample was collected on June from a lake (Pieczyska beach, Koronowo, Poland) and stored in sealed bottles at 4 °C in their raw form without filtration, for a few days, until the moment of the analysis.

2.2. Instrumentation

Chromatographic analyses were performed using an Agilent 7890B (Agilent, Santa Clara, CA, USA), equipped with a split/splitless injector, multipurpose autosampler, and flame ionization detector (FID).

The GC was fitted with a ZB-5 column (Zebron, Phenomenex Inc., Torrance, CA, USA), 30 m × 0.25 mm × 0.25 µm, containing (5% phenyl)-methylpolysiloxane.

The injector port was held at 230 °C and used in the split mode using a split ratio of 5:1, and the injection volumes were 1 µL. The detector temperature was 250 °C. The GC oven temperature program started at 80 °C and increased to 240 °C at 8 °C/min, where it was held for 13 min.

The structural characterization of the nutshells was performed by using a Bruker ALPHA Fourier-transform infrared spectrophotometer (FT-IR) (Berlin, Germany), using an attenuated total reflection technique (4500–360 cm^{-1} wavelength range). The morphology of the biosorbent was evaluated by scanning electron microscopy (SEM) using a LEO Electron Microscopy Ltd. 1430 VP (Cambridge, UK).

2.3. Preparation of the Biosorbent Material

Walnuts, hazelnuts, peanuts and pistachios were obtained from supermarket, Bydgoszcz, Poland. Firstly, the shells were separated from the nuts, and the materials were washed abundantly with tap water at room temperature. The wet shells were placed in

a laboratory dryer for 12 h at 80 °C. Then, the nutshells were ground (Polymix PX MFC 90D, Kinematica, Switzerland) to obtain a particle size of 800–500 µm (~55%) and <500 µm (~45%). Part of the material was ground (Grindomix GM 200, Retsch, Germany) to smaller particles of 500–200 µm (~50%) and <200 µm (~50%). Lastly, 5 g of prepared shell powder was placed in a beaker, and the material was washed with hot water (~80 °C) until colorless water was obtained. Next, the shells were washed with 5 mL of EA, and placed in a laboratory dryer for 12 h at 80 °C. The prepared biosorbents were stored in closed dark bottles.

2.4. Extraction Procedure

The extraction procedure is depicted in Figure 1. The extraction step was performed in a beaker containing 200 mg of biosorbent, and 10 mL of sample adjusted at pH 4 and 20% *w*/*v* of NaCl for 10 min. After that, the biosorbent was separated from the solution using empty SPE cartridges and SPE vacuum manifold. The biosorbent was dried by air (5 min). Then, material was immersed in the 750 µL of ACN/OE mixture (1:1, *v*:*v*) for 2.5 min, and the desorption step was repeated. The obtained extract was subjected to by nitrogen steam until evaporation (15 min). Then, 0.2 mL of ACN/OE mixture (1:1, *v*:*v*) containing IS was added to solution residue, and the extract was subjected to the GC analysis.

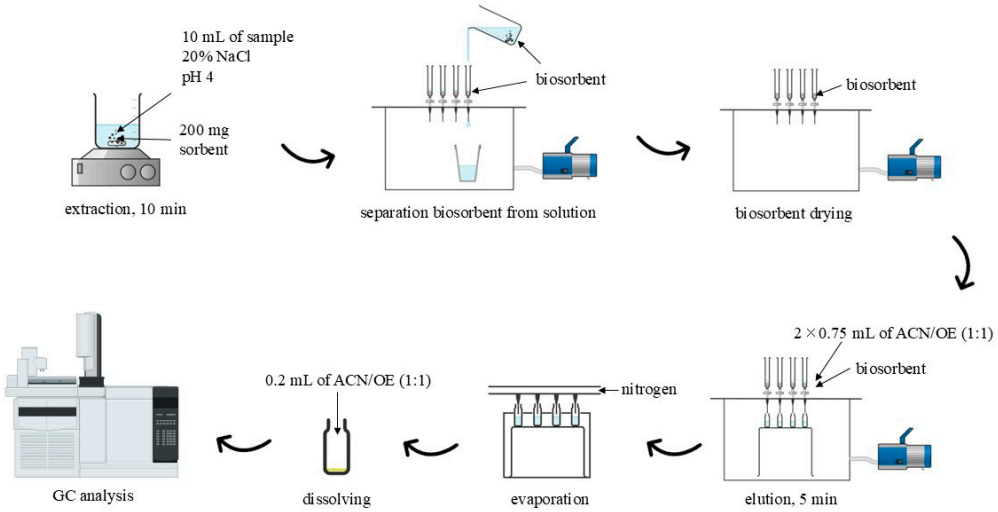


Figure 1. Scheme of the proposed method.

2.5. Method Validation

The analyte relative recovery and the intra- and inter-day precisions were determined by the analysis of lake samples spiked at three concentrations: 50, 200, and 500 µg/L. Precision was calculated as the relative standard deviation (RSD), considering a precision of less than 20% as the acceptance criterion. Repeatability was assessed by performing three determinations in one day for each concentration level. Intermediate precision was assessed by three determinations on another day for the medium concentration level (200 µg/L). Accuracy was evaluated as the percentage of recovery considering an acceptance criterion of 60–120% [22,23]. The experiments were performed in triplicate and the analysis was repeated at least three times.

3. Results and Discussion

3.1. Characterization of Biosorbents

Fourier transform infrared (FT-IR) spectroscopy was used to identify the main functional groups present on the four biosorbents' surfaces. Due to the fact that the obtained spectra are similar to each other, only the spectrum of the walnut shell is shown in Figure 2. All tested materials showed common peaks associated with the main components present in

nutshells: cellulose, hemicellulose, and lignin. An O-H stretching broad band at 3318 cm^{-1} and two sharpened bands at 2913 and 2879 cm^{-1} were observed in the C-H and C-H_2 stretching region—these bands are assigned to the methyl and methylene groups from lignin, hemicellulose, and cellulose. The intense C=O stretching band at 1731 cm^{-1} corresponds to the acetyl and ester groups in hemicellulose. The peak observed at 1654 and 1593 cm^{-1} is associate with the C=C group in aromatic groups of hemicellulose and lignin. The aromatic regions at 1504 and 1455 cm^{-1} correspond to lignin. The absorption peak at 1370 cm^{-1} is associated with the C-H group from the lignin methoxy groups, while C-C aromatic bonds are verified at 1326 cm^{-1}. The peaks at 1231 and 1156 cm^{-1} correspond to the C-O, and C-O-C stretching vibrations. The peak observed at 1028 cm^{-1} include contributions from lignin methoxy groups, cellulose, and hemicellulose ester groups. The infrared spectrum obtained is very similar to other spectra observed for nutshells in the literature [11,13,24].

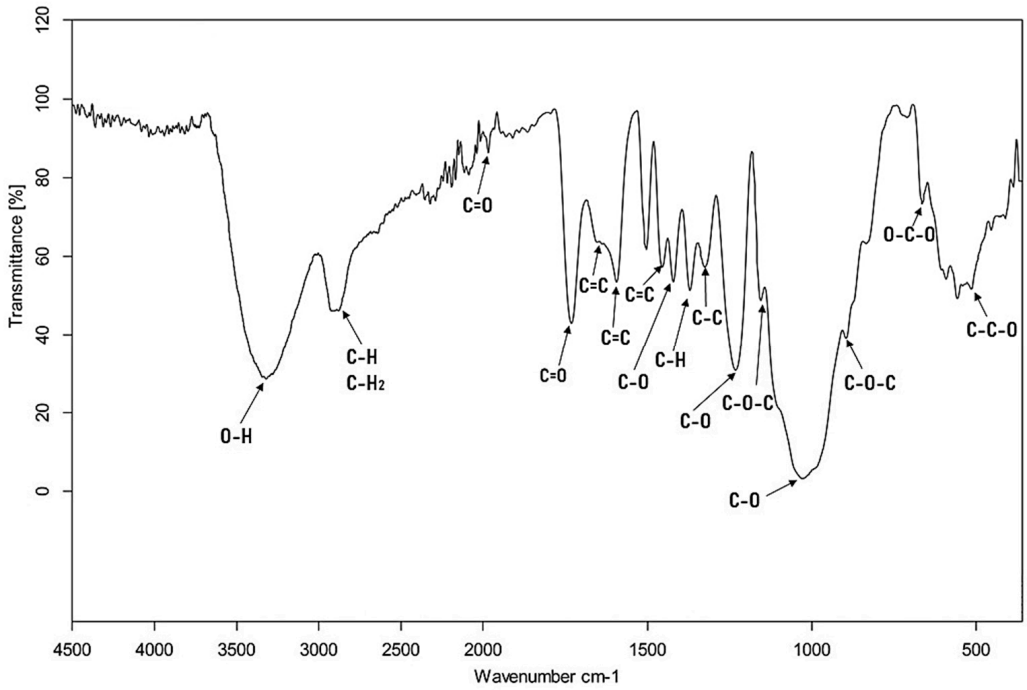


Figure 2. FT-IR spectrum of the walnut shells.

Scanning electron microscopy (SEM) was used to study the morphology of biosorbents, and the exemplary SEM micrographs before and after the sorption process using/for walnut and peanut shells are shown in Figure 3. The adsorbents exhibit irregular, rough, and porous structures of various shapes and sizes. Such structures are characteristic of lignocellulosic sorbents. As can be seen in Figure 3b,d after the sorption process, the sorbent structure became thicker and more folded. It may indicate the physical adsorption through adhesion of the analytes in the pores and on the surface of the biosorbent.

3.2. Preliminary Research

Methods of preparing an aqueous sample for chromatographic analyses using a biosorbent as a loose sorption bed are known from the literature. They consist, among others, of placing the sorbent in the pipette tip [25], and placing the sorbent in an empty SPE column [26]. After performing test analyses using these methods for the extraction of parabens and benzophenones, satisfactory results were not obtained. Expecting better extraction efficiency, it was decided to extend the contact time of the biosorbent with the analytes (the proposed method). As expected, the results at the initial stage were much better. The proposed procedure for sample preparation (extraction and desorption, shown in Figure 1) required the optimization of the sorption and desorption stages.

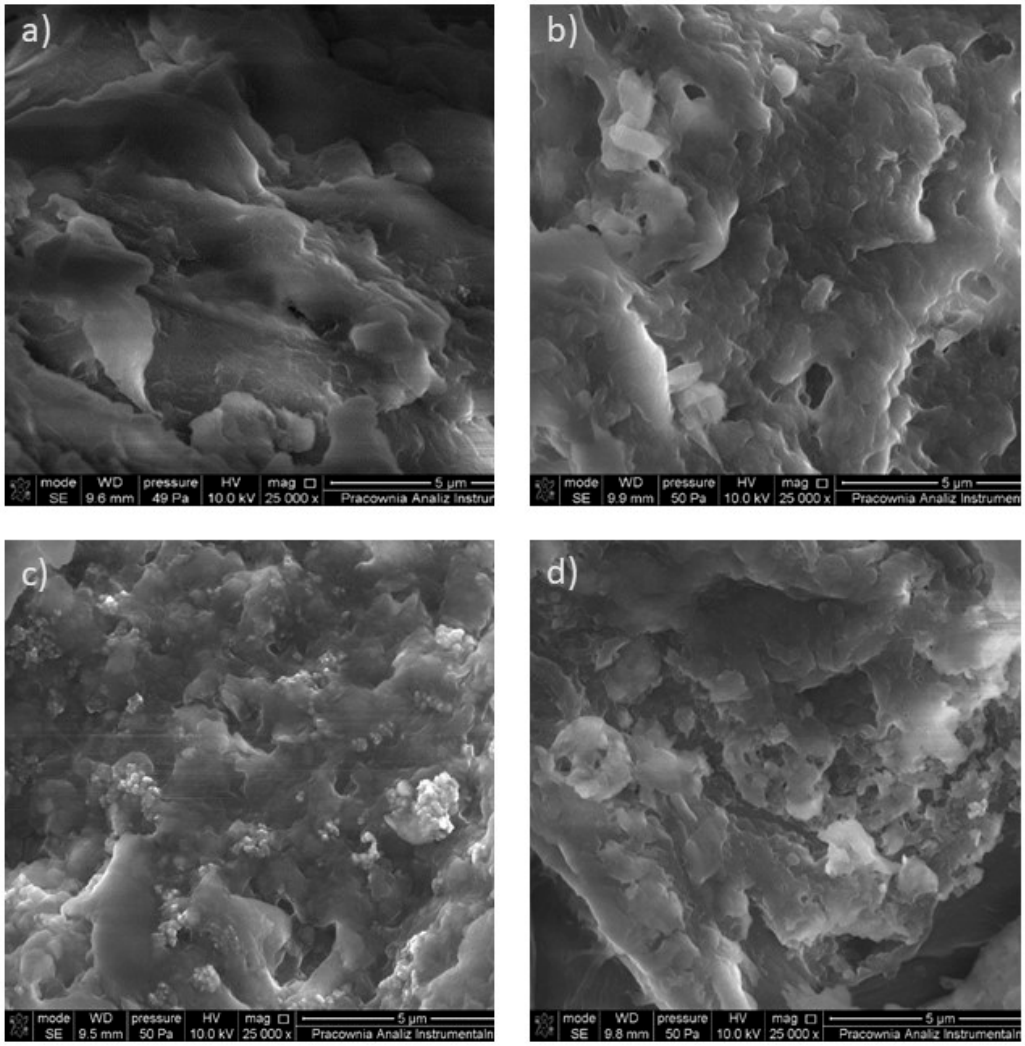


Figure 3. SEM images of (**a**) walnut shells; (**b**) walnut shells after adsorption of analytes; (**c**) peanut shells; (**d**) peanut shells after adsorption of analytes.

Preliminary studies were also conducted to determine the method of purifying the biosorbent. Different amounts of hot water (100–500 mL) and solvent (EA) (1–10 mL) were used for this purpose. Satisfactory results were obtained using 250 mL of hot water and 5 mL of EA for the Optimization of the proposed procedure.

3.3. Optimization of the Proposed Procedure

Several factors affecting the extraction efficiencies of the proposed method were tested, including the amount of biosorbent, the pH of the solution, the salting-out effect, the extraction time, the kind and volume of desorption solvent, the number of desorption cycles, and the desorption time. A mixed benzophenones and parabens standard containing 500 µg/L of each analyte was used to examine the extraction efficiency of the method. Walnut shells as biosorbents with a particle size of 800–500 µm (~55%) and <500 µm (~45%) were used for the optimization studies. The optimized parameters of the analytical method obtained using walnut shells were applied to determine the extraction efficiency for other shells. All optimization experiments were carried out in triplicate (n = 3).

3.3.1. Optimization of Biosorbent Mass

In order to obtain the highest possible amounts of extracted analytes, different masses of biosorbent ranging from 50 to 400 mg were evaluated for the extraction of the tested

analytes. As can be seen in Figure 4a, the best results were obtained using the biosorbent at a dose of 200 and more mg. Therefore, 200 mg of the sorbent was selected for further tests.

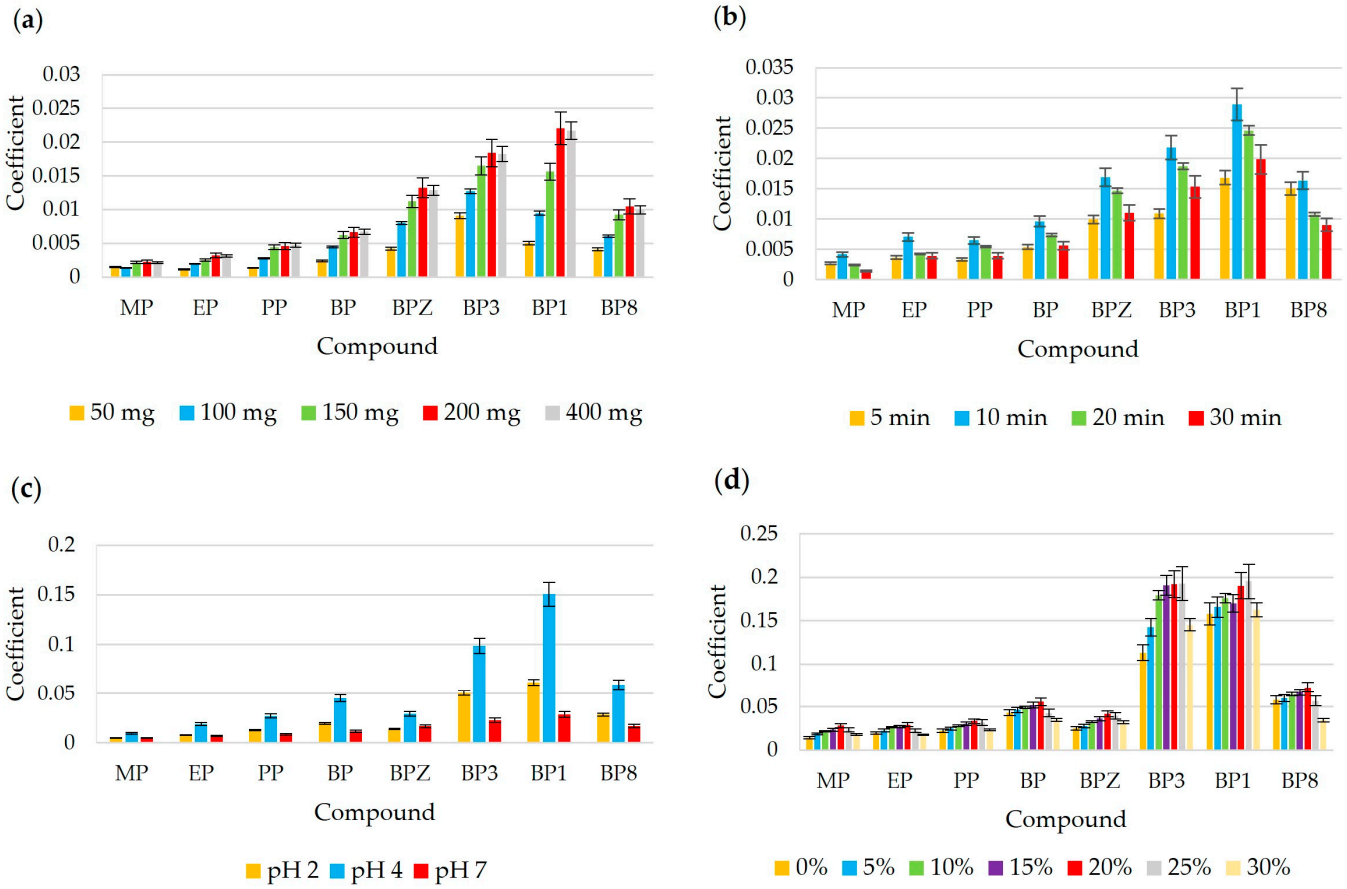


Figure 4. A bar graph for the optimization of (**a**) biosorbent mass: 10 mL of sample, pH 7, 20 min; (**b**) extraction time: 10 mL of sample, pH 7, biosorbent mass 200 mg; (**c**) pH solution: 10 mL of sample, 10 min, biosorbent mass 200 mg; (**d**) salt addition: 10 mL of sample, pH 4, 10 min, biosorbent mass 200 mg. For all optimization tests, the desorption step was performed with 1 × 1500 µL of ACN for 15 min.

3.3.2. Optimization of Extraction Time

As expected, it was shown that extraction time is an important parameter which influences the effectiveness of the analytes extracted. The effect of extraction time was evaluated in the range from 5 to 30 min. Figure 4b illustrates the effect of extraction time on the coefficient of the analytes. The extraction efficiency increased with increasing time, up to 10 min, and thence began to decrease. This could be due to the redissolution of the analyte into the sample solution. These results show that 10 min is enough for the complete equilibration of the analytes and biosorbent.

3.3.3. Optimization of pH

The pH value is important because it affects the ionization state and solubility of analytes in water. In this experiment, different sample solutions containing benzophenones and parabens with varying pH, namely 2, 4, and 7. The highest extraction efficiency for the proposed method was achieved at pH 4, where an approximately 100% increase in coefficient was achieved with respect to pH 7. For the efficient extraction of benzophenones and parabens ($pK_a \geq 7$), the pH of the sample solution should be lower than the pK_a of the analytes in order to obtain the target analytes in non-ionized forms, so that they

have a greater tendency to partition into the organic phase. The results are summarized in Figure 4c.

3.3.4. Optimization of Salting-Out Effect

The ionic strength adjustment by the salt addition was an also important parameter that could affect the extraction of the analytes. In the case of polar analytes (log K_{ow} < 4), the addition of NaCl, which increases the ionic strength, causes the hydration of salt ions, making water less accessible to organic compounds, promoting their migration towards the sorbent phase and reducing the solubility of analytes. The effect of ionic strength was studied by the addition of various amounts of NaCl (in the range from 0 to 30% w/v) to sample solutions. The extraction efficiency increases with increasing NaCl from 0 to 20%, and then decreases (Figure 4d). A decrease in salt contents greater than 20% may be due to an increase in solution viscosity, which in turn reduces the extraction kinetics. Therefore, the 20% addition NaCl to the sample solution was chosen as the optimal amount.

3.3.5. Optimization of the Desorption Step

The next relevant step of the optimization method was assigning the conditions of the desorption. Due to the fact that the tested analytes are polar (log $K_{o/w}$ < 4), a polar solvent was used for desorption of the analytes. Based on previous experiences and literature data, a polar solvent was selected for desorption, containing methanol (MeOH; log $K_{o/w} = -0.77$), ethyl acetate (OE; log $K_{o/w} = 0.73$), and acetonitrile (ACN; log $K_{o/w} = -0.34$). The highest extraction efficiency, especially for BP1, BP3 and BP8, was achieved when mixture of ACN/OE (1:1, v:v) was used as the desorption solvent. Thus, it was selected as the optimal desorption solvent (Figure 5a). Moreover, the volume and number of cycles of desorption solvent (ACN/OE mixture) was studied. For this purpose, 0.5, 1.0 and 1.5 mL of desorption solvent (ACN/OE mixture) was used. In order to be able to compare the results, the obtained coefficients were correlated with each other. It can be seen that using cyclic desorption gives better results for both the 1000 µL and 1500 µL volumes. The best response was obtained using 1.5 mL (2 timesfor 750 µL), and it was selected as the optimum volume for the desorption solvent (Figure 5b). In addition, desorption times of 1, 3, 5, 15, 20, and 30 min were evaluated and the best results was achieved with 5 min (Figure 5c).

3.3.6. Optimization of the Biosorbent Size

The last step of the optimization tests was to check how the size of biosorbent particles affects the extraction of analytes. For this purpose, two sizes of particles of nutshells were used: small particles (500–200 µm (~50%) and <200 µm (~50%)) and large particles (800–500 µm (~55%) and <500 µm (~45%)). Based on the obtained results, it can be concluded that more effective extraction was obtained using smaller biosorbent particles for all tested shells. For the walnut, hazelnut and peanut shells, smaller particles performed better than larger particles by 1 to 19%. For the pistachio shells, smaller particles gave better results by 1 to 40%, including 1–17% for benzophenones and 17–40% for parabens, as shown in Figure 5d. Smaller particles absorb analytes better because of their greater access to active sites on external surfaces and within pores.

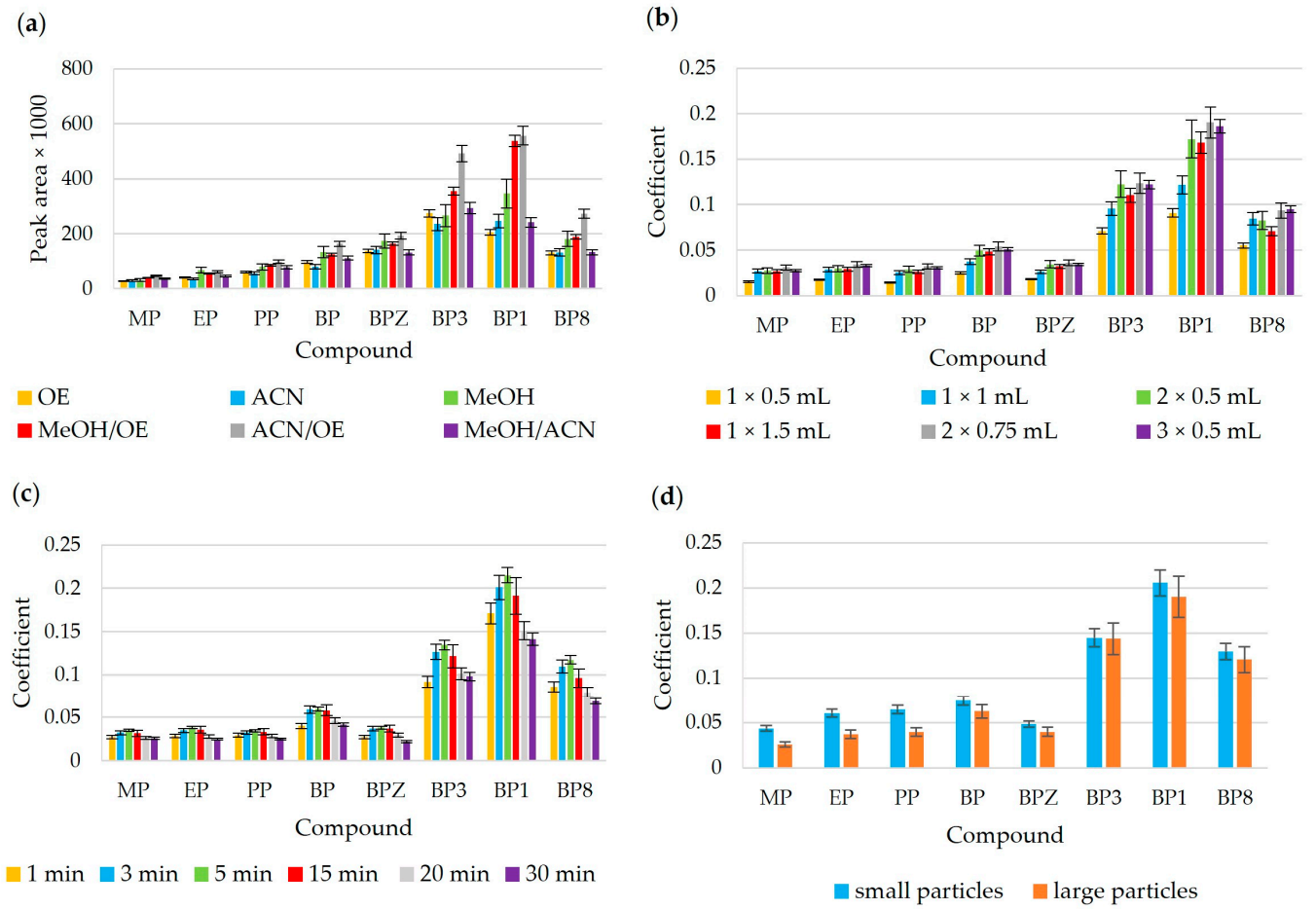


Figure 5. A bar graph for the optimization of the desorption condition: (**a**) the kind of desorption solvent: 1 × 1500 µL of solvent, 15 min; (**b**) the volume of solvent and number of cycles: desorption with ACN/OE, 15 min; (**c**) desorption time: 2 × 750 µL of ACN/OE; (**d**) biosorbent size: desorption with 2 × 750 µL of ACN/OE, 5 min. For all optimization tests, the sorption step was performed with 200 mg of biosorbent and 10 mL of sample, pH 4, 20% NaCl for 10 min.

3.4. Analytical Figures of Merit

The accuracy and repeatability of the proposed method using the nutshells as sorbent were evaluated by spiking the real samples (lake water sample) with analytes at concentration levels of 50, 200, and 500 µg/L. The recoveries ranged from 14 to 75% for parabens and from 59 to 117% for benzophenones (Table 2). It can be seen that in the developed method satisfactory recoveries (>60%) were obtained only for benzophenones. Particularly high recoveries (≥70%) was obtained for BP1, BP3, and BP8. The highest recovery rates for benzophenones were obtained using peanut shells (84–117%) and pistachio shells (87–101%). While, the RSDs of the procedure were satisfactory for both groups of analytes and the repeatability was lower than 20%.

Table 2. Analyte relative recoveries and intra- and inter-day precision in the lake samples (n = 3).

Analyte	Spiked Concentration (µg/L)	Walnut Shells			Hazelnut Shells			Peanut Shells			Pistachio Shells		
		RR (%)	RSD, Intra-Day (%)	RSD, Inter-Day (%)	RR (%)	RSD, Intra-Day (%)	RSD, Inter-Day (%)	RR (%)	RSD, Intra-Day (%)	RSD, Inter-Day (%)	RR (%)	RSD, Intra-Day (%)	RSD, Inter-Day (%)
MP	50	23	14.7	15.1	25	11.6	12.8	26	14.3	11.6	28	15.6	15.9
	200	16	15.6	18.9	18	11.2	17.4	14	9.7	12.6	16	18.6	14.6
	500	20	18.2	13.1	20	16.9	14.2	33	20.0	17.6	32	11.3	13.6
EP	50	28	11.2	12.8	26	12.3	11.2	53	9.6	11.3	49	12.9	12.3
	200	16	10.3	12.3	19	9.6	10.5	40	14.5	9.7	39	9.0	12.1
	500	33	16.1	11.0	28	18.4	17.4	52	12.3	14.3	46	14.7	9.6
PP	50	36	13.3	14.8	34	11.6	12.6	58	8.9	10.2	61	16.3	11.2
	200	20	9.8	12.6	27	8.3	9.6	41	4.3	7.3	57	4.0	6.9
	500	35	13.3	9.0	42	14.7	11.7	60	10.6	12.3	57	16.9	10.3
BP	50	49	8.2	11.8	48	11.6	18.5	69	16.3	17.6	67	14.0	11.3
	200	37	17.1	16.7	42	7.8	9.6	57	13.2	14.9	55	12.3	10.3
	500	53	14.3	12.8	47	14.2	11.2	75	11.8	12.8	73	8.6	9.3
BPZ	50	41	12.6	11.9	48	16.9	13.6	60	6.9	7.3	61	7.9	9.8
	200	49	15.7	18.3	52	12.4	10.5	66	3.5	9.8	68	12.3	9.8
	500	50	14.7	12.7	51	11.6	11.9	64	11.3	10.3	65	15.6	11.2
BP3	50	91	16.9	14.6	90	9.4	10.8	98	9.1	11.2	96	14.9	10.3
	200	87	2.9	9.3	86	10.3	13.6	84	19.3	17.6	91	10.2	9.8
	500	86	11.6	13.9	81	12.3	14.9	117	15.6	14.3	95	9.6	9.9
BP1	50	76	15.9	12.8	73	16.7	15.9	110	13.6	11.2	101	11.3	10.6
	200	69	0.8	7.9	69	15.6	13.8	102	7.8	10.9	95	8.7	9.3
	500	69	14.7	12.7	67	6.8	9.3	108	12.3	10.3	89	12.6	11.0
BP8	50	84	5.9	8.9	88	11.2	10.8	105	16.9	15.6	94	15.7	14.7
	200	80	16.4	14.8	82	14.3	16.9	101	12.6	14.9	87	5.0	9.0
	500	73	11.2	9.6	90	9.8	10.4	102	11.3	13.6	88	16.3	15.2

The enrichment factors (EFs), calculated from the ratio of the extracted analyte concentration in the solvent phase to the initial concentration in the aqueous phase, were found to be in the range of 7–37.5 for parabens and 19.5–58.5 for benzophenones.

The proposed method was applied in the determination of parabens and benzophenones in lake water samples and the concentrations of the analytes were all below the quantification limits (LOQ: 30–90 µg/L for parabens and 15–33 µg/L for benzophenones).

3.5. Greenness Assessment

The proposed sample preparation method was evaluated using the Analytical Greenness metric for sample preparation (AGREEprep); the metric was introduced in 2022 by Wojnowski et al. [27]. In the AGREEprep metrics, the sample preparation method is assigned a score related to using the solvents, materials and reagents, waste generation, energy consumption, sample amount, and throughput. Each part has a score of 0–1, and the proximity to 1 indicates the greenness of the method. A method that achieves a total score greater than 0.5 is classified as a "green" method. The pictogram shown in Figure 6 was obtained as a result of using the AGREEprep metric tool software, evaluating the ten assessed categories and the total assessment, which is 0.54. A summary of the aspects considered in each category is detailed in the generated report (Supplementary Material) with calculated score values.

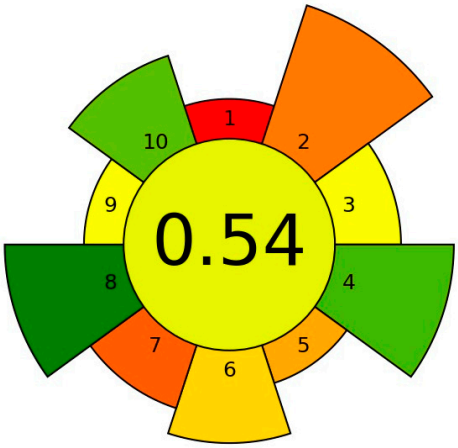


Figure 6. An assessment of greenness of the proposed sample preparation method, obtained by using the AGREEprep tool.

The lowest scores were obtained in categories 1 and 7, which concerned (principle 1) the location of sample preparation (the need to perform tests in a laboratory) and the aspects of "*integration and automation*" (principle 7)—the procedure requires three steps (extraction, elution and evaporation). Nevertheless, the greenest advantages of the proposed method were related to the low waste generation (principle 4), estimated as 0.4 mL per sample, the low energy consumption (3 Wh per sample, principle 8), and the "*operator's safety*" (principle 10), which only involves one hazard related to the consumption of ACN and OE as solvents. Taking into account the obtained evaluation results, it can be stated that the proposed method can be classified as a "green" sample preparation method.

3.6. Comparison Proposed Method with Other Methods

The efficiency of proposed procedure was evaluated by comparing it with other methods [25,26,28–31] which use biosorbents for the determination of UV filters and parabens in water samples (Table 3). Among the mentioned methods, the bar adsorptive microextraction (BAµE) was most frequently used; however, in comparison to the method proposed by us, it uses a larger sample volume, and the extraction of analytes takes much longer (>45 min). In general, when comparing the developed method with other methods, a similar analytical performance was obtained in relation to precision and recoveries. Additionally,

the sensitivity of the proposed method may be lower when a different detector (e.g., MS, MS/MS) is used.

Table 3. Comparison of proposed method with other methods reported in the literature.

Analytes [a]	Biomaterial	Matrix	Technique [b]	Instrumentation	Sample Volume	Solvent for Desorption (Volume)	Extraction Time	Recovery (%)	Ref.
MP, EP, PP, BP	cork	river water	BAμE	HPLC-DAD	15 mL	MeOH and ACN (120 μL)	45 min	53–124	[28]
MP, EP, BPZ	diatomaceous earth	lake water	BAμE	HPLC-DAD	15 mL	MeOH and ACN (100 μL)	90 min	63–124	[29]
MP, PP, BP, BPZ	*Araucaria angustifolia* bracts	river water	BAμE	HPLC-DAD	30 mL	ACN and water (80 μL)	180 min	62–115	[30]
MP, EP, BPZ, 4-MBC, OD-PABA	cork	lake water	DPX	HPLC-DAD	800 μL	MeOH and ACN (100 μL)	90 s	71–132	[25]
ES, EDP, IMC, OCR, EMC, 4-MBC, HS	cellulose	spiked water	disk-SPE	HPLC-UV-Vis	20–100 mL	2-propanol (4500 μL)	-	60–70	[26]
4-MBC, OD-PABA	cork	river water	SPME	GC-MS	25 mL	-	70 min	67–107	[31]
MP, EP, PP, BP	walnut, hazelnut, peanut, pistachio shells	lake water	-	GC-FID	10 mL	ACN and OE (1500 μL)	10 min	14–75	this work
BPZ, BP1, BP3, BP8								59–117	

[a] OD-PABA: 2-ethylhexyl 4-(dimethylamino)benzoate; 4-MBC: 3-(4-methylbenzyli-dene)camphor; OCR: octocrylene; EMC: 2-ethylhexyl 4-methoxycinnamate; MBC: 3-(4-methylbenzyli-dene)camphor; HS: homosalate; ES: 2-Ethylhexyl salicylate; EDP: 2-ethylhexyl 4-(dimethylamino)benzoate (ethylhexyl dimethyl PABA); IMC: isoamyl 4-methoxycinnamate. [b] DPX: disposable pipette extraction; SPME: solid-phase microextraction; BAμE: bar adsorptive microextraction.

4. Conclusions

Nutshells are one of the wastes produced by the food industry and have been proven to be an economical substitute sorbent for the identification and quantification of benzophenones and parabens in water. The developed analytical method, for the first time, uses nutshells such as hazelnut, walnut, peanut and pistachio shells to determine the above-mentioned analytes in a water matrix. Nutshells have proven to be an effective natural sorbent that can retain the ingredients of personal care products from the aqueous matrix. According to the principles of green sample preparation, this method is characterized by low organic solvent consumption, low energy consumption, simplicity, speed, miniaturization and the use of a safe, sustainable, renewable and biodegradable sorbent. Therefore, it is environmentally friendly according to the so-called Green Analytical Chemistry, which was confirmed by the AGREEprep tool. Good enrichment factors, high relative recovery and other satisfactory analytical results were obtained, in particular for benzophenones. Studies show that nutshells are an alternative sorption material for commercially available sorbents. However, they require further intensive research on the possibility of their wider use in analytical chemistry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ma17205128/s1, Supplementary Material S1: AGREEprep report.

Author Contributions: Conceptualization, I.N. and G.W.; methodology, I.N. and P.K.; validation, I.N. and G.W.; formal analysis, I.N. and G.W.; writing—original draft preparation, I.N. and G.W.; writing—review and editing, I.N., G.W. and P.K.; visualization, I.N. and G.W.; supervision, G.W. and P.K. All authors have read and agreed to the published version of the manuscript.

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Nutshell Materials as a Potential Eco-Friendly Biosorbent for the Effective Extraction of UV Filters and Parabens from Water Samples

Izabela Narloch , Gra˙zyna Wejnerowska * and Przemysław Kosobucki

AGREEprep

Analytical Greenness Metric
for Sample Preparation

* evaluation for simultaneous preparation of 6 samples

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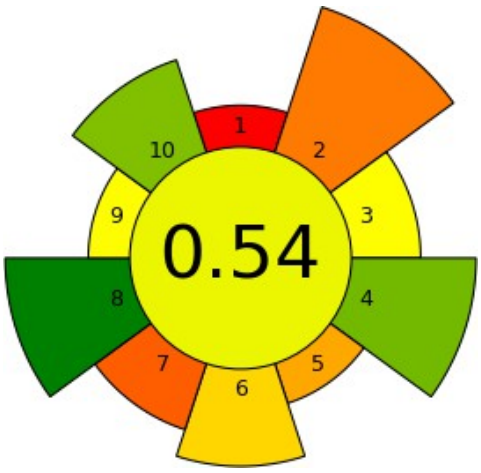

#	Criterion	Score	Weight
1.	**Sample preparation placement** Sample preparation placement: Ex situ	0.0	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 1.9	0.24	5
3.	**Sustainability and renewability of materials** 50-75% of reagents and materials are sustainable or renewable and can only be used once	0.5	2
4.	**Waste** Mass [g] or volume [mL] of waste: 0.4	0.78	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 10	0.33	1
6.	**Sample throughput** Hourly sample throughput: 6	0.42	3
7.	**Integration and automation** No. of sample prep. steps: 3 steps; degree if automation: Manual systems	0.19	2
8.	**Energy consumption** Approximate energy consumption per analysis [W]: 3	1.0	4
9.	**Post-sample preparation configuration for analysis** GC with non-MS detection, atomic absorption spectroscopy, capillary electrophoresis, etc.	0.5	1
10.	**Operator's safety** No. of distinct hazards: 1 hazard	0.75	3

7.2. OŚWIADCZENIE AUTORA ROZPRAWY DOKTORSKIEJ

Oświadczenie Autora rozprawy doktorskiej

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OŚWIADCZENIE

Oświadczam, iż mój wkład autorski w niżej wymienionych artykułach naukowych stanowiących cykl publikacji rozprawy doktorskiej był następujący*:

1. **Narloch Izabela**, Wejnerowska Grażyna, An overview of the analytical methods for the determination of organic UV filters in cosmetic products and human samples, Molecules (MDPI), 2021, 26, 4780, https://doi.org/10.3390/molecules26164780, 140 pkt. MNiSW, IF = 4.2.
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2. Wejnerowska Grażyna, **Narloch Izabela**, Determination of benzophenones in water and cosmetics samples: a comparison of solid-phase extraction and microextraction by packed sorbent methods, Molecules (MDPI), 2021, 26, 6896, https://doi.org/10.3390/molecules26226896, 140 pkt. MNiSW, IF = 4.2.
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   e) udział w analizie i interpretacji wyników prac badawczych,
   f) udział w opracowaniu manuskryptu,
   g) udział w opracowaniu odpowiedzi na recenzje.

3. **Narloch Izabela**, Wejnerowska Grażyna, Comparison of the effectiveness and environmental impact of selected methods for the determination of fatty acids in milk samples, Molecules (MDPI), 2022, 27, 8242, https://doi.org/10.3390/molecules27238242, 140 pkt. MNiSW, IF = 4.2.
   Wykonane zadania przez Doktoranta w ramach artykułu:
   a) udział w opracowaniu koncepcji manuskryptu,
   b) przeprowadzenie przeglądu literatury naukowej,
   c) udział w planowaniu metodologii prac badawczych,
   d) przeprowadzenie prac badawczych,
   e) udział w analizie i interpretacji wyników prac badawczych,

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* *W przypadku prac dwu- lub wieloautorskich wymagane są oświadczenia kandydata do stopnia doktora oraz współautorów, wskazujące na ich merytoryczny wkład w powstanie każdej pracy (np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań – np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie ankiet itp., wykonanie analizy wyników, przygotowanie manuskryptu artykułu i inne). Określenie wkładu danego autora, w tym kandydata do stopnia doktora, powinno być na tyle precyzyjne, aby umożliwić dokładna ocenę jego udziału i roli w powstaniu każdej pracy.*

f) wykonanie analizy statystycznej,
g) udział w opracowaniu manuskryptu,
h) udział w opracowaniu odpowiedzi na recenzje,
i) edycja końcowa manuskryptu.

4. **Narloch Izabela**, Wejnerowska Grażyna, A comparative analysis on the environmental impact of selected methods for determining the profile of fatty acids in cheese, Molecules (MDPI), 2023, 28, 4981, https://doi.org/10.3390/molecules28134981, 140 pkt. MNiSW, IF = 4.2.
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a) udział w opracowaniu koncepcji manuskryptu,
b) przeprowadzenie przeglądu literatury naukowej
c) udział w planowaniu metodologii prac badawczych,
d) przeprowadzenie prac badawczych,
e) udział w analizie i interpretacji wyników prac badawczych,
f) wykonanie analizy statystycznej,
g) udział w opracowaniu manuskryptu,
h) udział w opracowaniu odpowiedzi na recenzje,
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5. Wejnerowska Grażyna, **Narloch Izabela**, Comparison of the greenness assessment of chromatographic methods used for analysis of UV filters in cosmetic samples, Analytica (MDPI), 2023, 4, 447-455. https://doi.org/10.3390/analytica4040032, 5 pkt. MNiSW, IF = 0.
Wykonane zadania przez Doktoranta w ramach artykułu:
a) udział w opracowaniu koncepcji manuskryptu,
b) przeprowadzenie przeglądu literatury naukowej,
c) udział w analizie i interpretacji ocen ekologiczności metod,
d) udział w opracowaniu manuskryptu,
e) udział w opracowaniu odpowiedzi na recenzje.

6. **Narloch Izabela**, Wejnerowska Grażyna, A comprehensive assessment of sample preparation methods for the determination of UV filters in water by gas chromatography-mass spectrometry: greenness, blueness, and whiteness quantification using the AGREEprep, BAGI, and RGB 12 tools, Applied Sciences (MDPI), 2024, 14, 7690, https://doi.org/10.3390/app14177690, 100 pkt. MNiSW, IF = 2.5.
Wykonane zadania przez Doktoranta w ramach artykułu:
a) udział w opracowaniu koncepcji manuskryptu,
b) przeprowadzenie przeglądu literatury naukowej,
c) udział w analizie i interpretacji ocen ekologiczności metod,
d) udział w opracowaniu manuskryptu,
e) udział w opracowaniu odpowiedzi na recenzje.

7. **Narloch Izabela**, Wejnerowska Grażyna, Kosobucki Przemysław, Nutshell materials as a potential eco-friendly biosorbent for the effective extraction of UV filters and parabens from water samples, Materials (MDPI), 2024, 17, 5128, https://doi.org/10.3390/ma17205128, 140 pkt. MNiSW, IF = 3.1.
Wykonane zadania przez Doktoranta w ramach artykułu:
a) udział w opracowaniu koncepcji manuskryptu,
b) przeprowadzenie przeglądu literatury naukowej,
c) przeprowadzenie prac badawczych,
d) udział w analizie i interpretacji wyników prac badawczych,
e) udział w opracowaniu wyników badań dotyczących charakterystyki sorbentów,

f) udział w opracowaniu manuskryptu,
g) udział w opracowaniu odpowiedzi na recenzje,
h) edycja końcowa manuskryptu.

Bydgoszcz, 16.05.2025
miejscowość, data

Izabela Narloch
Podpis Autora rozprawy doktorskiej

P. Koroluk
Podpis promotora

7.3. OŚWIADCZENIA WSPÓŁAUTORÓW ROZPRAWY DOKTORSKIEJ

Oświadczenie Współautora

dr hab. Przemysław Kosobucki, prof. PBŚ
(tytuł zawodowy, imiona i nazwisko współautora)

Politechnika Bydgoska im. Jana i Jędrzeja Śniadeckich
Wydział Technologii i Inżynierii Chemicznej
(miejsce pracy/afiliacja)

OŚWIADCZENIE

Oświadczam, iż mój wkład autorski w niżej wymienionym artykule naukowym był następujący*:

1. Narloch Izabela, Wejnerowska Grażyna, **Kosobucki Przemysław**, Nutshell materials as a potential eco-friendly biosorbent for the effective extraction of UV filters and parabens from water samples, Materials (MDPI), 2024, 17, 5128, https://doi.org/10.3390/ma17205128, 140 pkt. MNiSW, IF = 3.1.
   Wykonane zadania w ramach artykułu:
   a) nadzór merytoryczny nad realizacją badań,
   b) udział w opracowaniu wyników badań dotyczących charakterystyki sorbentów,
   c) udział w opracowaniu manuskryptu,
   d) udział w opracowaniu odpowiedzi na recenzje.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez mgr inż. Izabelę Narloch jako część rozprawy doktorskiej opartej na zbiorze opublikowanych i powiązanych tematycznie artykułów naukowych.

16.05.2025 Bydgoszcz
miejscowość, data

P. Kosobucki
podpis Współautora

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* *W przypadku prac dwu- lub wieloautorskich wymagane są oświadczenia kandydata do stopnia doktora oraz współautorów, wskazujące na ich merytoryczny wkład w powstanie każdej pracy (np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań – np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie ankiet itp., wykonanie analizy wyników, przygotowanie manuskryptu artykułu i inne). Określenie wkładu danego autora, w tym kandydata do stopnia doktora, powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej pracy.*

Oświadczenie Współautora

dr inż. Grażyna Wejnerowska
(tytuł zawodowy, imiona i nazwisko współautora)

Politechnika Bydgoska im. Jana i Jędrzeja Śniadeckich
Wydział Technologii i Inżynierii Chemicznej
(miejsce pracy/afiliacja)

OŚWIADCZENIE

Oświadczam, iż mój wkład autorski w niżej wymienionych artykułach naukowych był następujący*:

1. Narloch Izabela, **Wejnerowska Grażyna**, An overview of the analytical methods for the determination of organic UV filters in cosmetic products and human samples, Molecules (MDPI), 2021, 26, 4780, https://doi.org/10.3390/molecules26164780, 140 pkt. MNiSW, IF = 4.2.
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   a) udział w opracowaniu koncepcji manuskryptu,
   b) udział w opracowaniu manuskryptu,
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2. **Wejnerowska Grażyna**, Narloch Izabela, Determination of benzophenones in water and cosmetics samples: a comparison of solid-phase extraction and microextraction by packed sorbent methods, Molecules (MDPI), 2021, 26, 6896, https://doi.org/10.3390/molecules26226896, 140 pkt. MNiSW, IF = 4.2.
   Wykonane zadania w ramach artykułu:
   a) udział w opracowaniu koncepcji manuskryptu,
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   c) udział w planowaniu metodologii prac badawczych,
   d) udział w przeprowadzeniu prac badawczych,
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   b) udział w planowaniu metodologii prac badawczych,
   c) wykonanie ocen ekologiczności metod badawczych,
   d) udział w analizie i interpretacji wyników prac badawczych,
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c) wykonanie ocen ekologiczności metod badawczych,
d) udział w analizie i interpretacji wyników prac badawczych,
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d) udział w analizie i interpretacji ocen ekologiczności metod,
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Wykonane zadania w ramach artykułu:
a) udział w opracowaniu koncepcji manuskryptu,
b) wykonanie oceny ekologiczności metody badawczej,
c) udział w analizie i interpretacji wyników prac badawczych,
d) udział w opracowaniu manuskryptu,
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Bydgoszcz, 16.05.2025
miejscowość, data

podpis Współautora