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(Article begins on next page)

Portable voltammetry: a rapid and efficient technique for determining UV filters in cosmetics: a comparative study with HPLC-PDA and HPLC-MS/MS

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32 **ABSTRACT**

33

34 This study addresses the widespread use of UV filters (UVFs) in cosmetic and solar products due to the
35 negative effects of UV radiation, particularly in relation to melanoma risk. While these filters offer
36 protection, their extensive application raises concerns about their environmental and health impacts.
37 Organic UVFs, in particular, have been associated with endocrine disruption in aquatic species and coral
38 reef damage. To mitigate these concerns, regulatory limits have been imposed on certain UVFs. Current
39 analytical techniques for UVF determination, such as HPLC-PDA and HPLC-MS/MS, offer high accuracy but
40 are expensive and lack on-site monitoring capabilities. In response, this research aims to develop a rapid
41 and cost-effective method, utilizing voltammetry for organic UVF quantification in complex matrices like
42 sunscreens. Additionally, HPLC-PDA and HPLC-MS/MS are employed for electrochemical methods and
43 device validation. This approach not only addresses the need for efficient UVF analysis but also provides a
44 basis for regulatory compliance and environmental stewardship in the cosmetics industry.

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47 INTRODUCTION

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49 Melanoma is definitely the most common cancer of the skin, and among the causes is reported exposure
50 to UV rays. Protection from these rays could be reached using filters that reduce the exposure or
51 absorption, and this option is common in the cosmetic industry. In fact, many cosmetics report sunscreen
52 inside.¹ UV filters (UVFs) are substances added to different cosmetic products such as sunscreen, lip balm,
53 hair spray, and shampoo both to protect the skin from the possible harmful effects of UV radiation and to
54 improve the stability and durability of the product.^{2,3} In addition, UVFs are also added in plastics, paints,
55 polymeric materials, and textiles to prevent sunlight-induced photodegradation and to prevent color
56 changes in materials.^{3,4} In sunscreen products, a mixture of UVFs is used both to protect the skin from the
57 entire range of harmful UV rays and to avoid the photodegradation of organic filters and preserve their
58 potential for protection.⁵ As European Union (EU) Regulation 1223/2009-Cosmetic Regulation reported,
59 UVFs are “substances which are exclusively or mainly intended to protect the skin against certain UV
60 radiation by absorbing, reflecting or scattering UV radiation”.⁶ Sunscreen products are characterized by
61 SPF (sun protection factor), a numerical index indicating the effective- ness of sunscreen that also depends
62 on the amount of UVFs present in the formulation and is determined by in vitro or in vivo testing. UVFs are
63 distinguished in chemical (organic) or physical (inorganic), and these differ in the mechanism of protection.
64 The former absorb UV-A (315–400 nm) and UV- B (280–315 nm) radiations and convert them into heat,⁷
65 while the latter block UV radiation by diffusion and reflection processes.^{5,8,9} In addition to the wide
66 pollution produced, chemical UVFs are considered possible endocrine disrupters for various animal
67 species.¹⁰ For example, prolonged exposure of fish to benzophenone-3 (BP3) has been shown to cause
68 endocrine disruption, inducing reproductive disease, reducing egg production and hatching, and
69 stimulating the production of vitellogenin protein in male fish.¹¹ UVFs also have negative impacts on coral
70 reefs,¹² and BP3 has been called a threat to coral reefs around the world. It is estimated that up to 14000
71 tons of sunscreen, some containing up to 10% BP3, are released every year in areas of the coral reef, and
72 this puts about 10% of global coral reefs and up to 40% of coastal reefs at the risk of coral bleaching.¹³
73 Thus, for these side effects of UVFs on human health and their possible bioaccumulation in animals and
74 waters, the European Commission established limits of concentration for UVFs, such as benzophenone-3
75 and octocrylene (that were indicated as endocrine disruptors), in order to reach a compromise between
76 accurate protection and minimal negative impact.^{6,14} Conventional analytical techniques such as high-
77 performance liquid chromatography coupled to photodiode array (HPLC-PDA) and high-performance liquid
78 chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) are mainly used for the
79 determination of chemical UVFs.^{15–17} These two analytical approaches make it possible to perform very

80 accurate analyses but are found to be expensive. Moreover, they are techniques that do not allow “on-
81 site” monitoring. The aim of this work is to develop a method and a portable device for the rapid
82 determination of organic UVFs in complex matrices such as sunscreen products, using square wave
83 voltammetry (SWV) as an analytical technique, allowing rapid, simple, and inexpensive quantification of
84 UVFs. In addition, HPLC-PDA and HPLC-MS/MS were used as comparison techniques to validate the
85 developed method and the portable device.

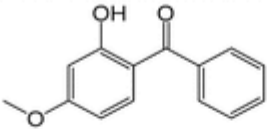
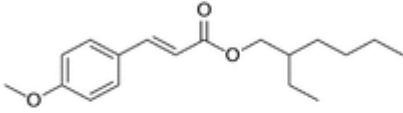
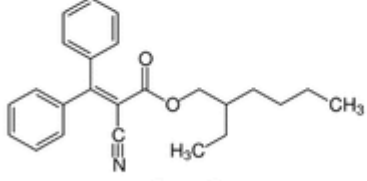
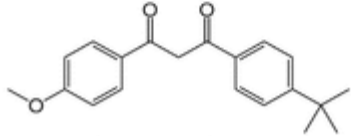
86 87 88 **EXPERIMENTAL SECTION**

89 90 **Chemicals and Reagents**

91 All chemicals were analytical reagent grade and used without further purification. High-purity water (HPW)
92 was used in all of the experiments and was obtained using an Elix Advantage reverse osmosis system and a
93 Millipore-Milli-Q Plus from Merck (Darmstadt, Germany). Certified UVF standards (Table 1) of octocrylene
94 (OC-CAS-No: 6197-30-4), benzophenone-3 (BP3-CAS-No: 131-57-7), ethylhexyl methoxycinnamate (EHMC-
95 CAS-No 5466-77-3), and butyl methoxydibenzoylmethane (BMDM-CAS-No: 70356-09-1) were purchased
96 from Sigma-Aldrich (Darmstadt, Germany) and were prepared by dissolving a known amount of standard
97 in ethanol. Methanol (CAS-No: 67-56-1) and ethanol (CAS-No: 64-17-5) were purchased from VWR
98 Chemicals (Milan, Italy). Sodium chloride (NaCl, CAS-No: 7647-14-5) was purchased from Sigma-Aldrich
99 (Darmstadt, Germany), while acetonitrile (CAS-No: 75-05-8) and formic acid (CAS-No: 64-18-6) were
100 purchased from Carlo Erba Reagent (Milan, Italy). Cetyltrimethylammonium bromide (CTAB, CAS-No: 57-
101 09-0) was purchased from Sigma-Aldrich (Darmstadt, Germany).

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Table 1. Certified UVFs

CHEMICAL NAME (OTHERS)	INCI (UV Protection)	STRUCTURE	ACRONYM
2-HYDROXY-4-METHOXYBENZOPHENONE (OXYBENZONE)	BENZOPHENONE-3 (UVA2 – UVB)		BP-3
2-ETHYLHEXYL 3-(4-METHOXYPHENYL)PROP-2-ENOATE (OCTINOXATE)	ETHYLHEXYL METHOXYCINNAMATE (UVA2 – UVB)		EHMC
ETHYLHEXYL 2-CYANO-3,3-DIPHENYL-2-PROPENOATE	OCTOCRYLENE (UVA1 – UVB)		OC
1,3-PROPANEDIONE, 1-[4-(1,1-DIMETHYLETHYL)PHENYL]-3-(4-METHOXYPHENYL) (AVOBENZONE)	BUTYL METHOXYDIBENZOYLMETHANE (UVA1)		BMDB

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Electrochemical Instrumentation

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The analyses were performed using an electrochemical cell consisting of a glass carbon electrode (GCE) as a working electrode (WE), a reference electrode (RE) Ag/AgCl filled with a solution of 3 M KCl, and a counter electrode (CE) in Pt from Metrohm s.r.l. (Origgio, Italy). The electrochemical cell is connected to a PalmSens3 portable potentiostat from Palmsens (Houten, Netherlands). PSTrace5.9 software allows us to set working conditions and view and process voltammograms. A solution consisting of 0.1 M NaCl, methanol, ethanol, and 0.30 mM CTAB was used as a supporting electrolyte. The voltammetric method used for the analysis is cathodic stripping voltammetry with square wave potential scanning (SWV). The procedure for the preparation of standards for electrochemical analysis is given in the Supporting Information (SI).

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Chromatographic Instrumentation

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Analyses were performed using an HPLC Thermo Fisher Scientific liquid chromatography system (Spectra System P2000) coupled to a photodiode array detector (PDA) (Spectra System UV6000LP). The mobile phase was degassed directly online by using a Biotech Degasi Classic (LabService, Italy). Excalibur v.2.0 Software (Thermo Fisher Scientific, Waltham, MA) was used to collect and analyze data. The Hypersil GOLD PFP (5 × 2.1 mm, 1.9 μm particle size; Thermo Fisher Scientific) column was

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123 used to separate UVFs. The column was thermostated at 30 °C (± 1 °C) by using a Jetstream2 Plus
124 column oven during the analysis. The mobile phase was a mixture of HPW (A) and acetonitrile (B)
125 both with 0.1% formic acid. The mobile phase composition was A/B = 52%:48% (v), and the analysis
126 was performed in isocratic mode at a flow rate of 0.4 mL/min. The entire chromatographic run was
127 performed in 25 min. The injection volume was set at 5 μ L. UVFs were detected at their maximum
128 wavelengths and at their respective retention times, as reported in the SI (Table S1) with the
129 chromatogram of the mixture of UVFs considered (Figure S1). In the SI, there are also the total
130 method validation figure of merits (see Tables S3 and S4 for intra- and interday analytical
131 parameters, and Table S5 for ruggedness). The possible adsorption of analytes on the surface of the
132 vials used has also been evaluated, as reported in SI Figure S2 and Table S6, with the aim of
133 choosing the best material to conduct the extraction procedure.

134 **Regarding the HPLC-MS/MS method**

135 Analyses were carried out on a Waters (Milford, MA) Alliance 2695 chromatographic system with an
136 autosampler coupled to a Waters Micromass Quattro Micro triple-quadrupole tandem mass
137 spectrometer, interfaced with an electrospray ion source working in positive and negative ionization
138 modes (ESI+/ESI-) with polarity switching. Data processing was performed using Waters MassLynx
139 4.1 software. Chromatography was obtained by exploiting the same combination of stationary and
140 mobile phases as the HPLC-PDA system.

141 In order to develop an original HPLC-MS/MS method for the purpose, a multiple reaction
142 monitoring (MRM) method was set up exploiting exclusive m/z transitions for each analyte.
143 Parameter settings were optimized via direct infusion of individual analytes (1 μ g/mL methanolic
144 solutions) at 20 μ L/min. Optimized m/z transitions for each considered analyte, HPLC-MS/MS MRM
145 parameters, and method validation results are reported in Supplementary Table S7.

147 **Cosmetic Samples**

148 The cosmetic products considered in this study were purchased at local markets, trying to evaluate
149 all of the types of products and the main sun protection factors (SPFs) generally used. The table
150 containing all of the samples, the UVFs of interest, and the SPF are reported in the SI Table S8.

153 RESULTS AND DISCUSSION

154 155 **Optimizing Conditions for Voltammetric Analysis**

156 The objective of this work is to develop a voltammetric procedure for the determination of UVFs in
157 cosmetic products¹⁸. However, before analyzing real samples, it is necessary to assess the
158 instrumental response of UVFs using certified standards at known concentrations. In particular,
159 octocrylene (OC), benzophenone-3 (BP3), ethylhexyl methoxycinnamate (EHMC), and butyl
160 methoxydibenzoylmethane (BMDM) have been studied. Initially, UVFs were considered individually,
161 and after optimizing the operational parameters, the application of the technique for the
162 simultaneous determination of the analytes was also evaluated. Voltammograms were recorded by
163 using a square wave potential scan in the cathodic direction, capturing the signal corresponding to
164 their reduction at the electrode.

165 Different compositions of the supporting electrolyte were varied to obtain a linear background
166 signal with low currents. The solution for obtaining linear signals and low currents is 8 mL of 0.1 M
167 NaCl, 1.0 mL of ethanol, 1.0 mL of methanol, and 0.30 mM of CTAB. Subsequently, the instrumental
168 parameters of the SWV were optimized in order to improve instrumental sensitivity. Among the
169 different parameters, the parameter that most affects sensitivity is frequency. Table 2 shows the
170 optimized SWV parameters.

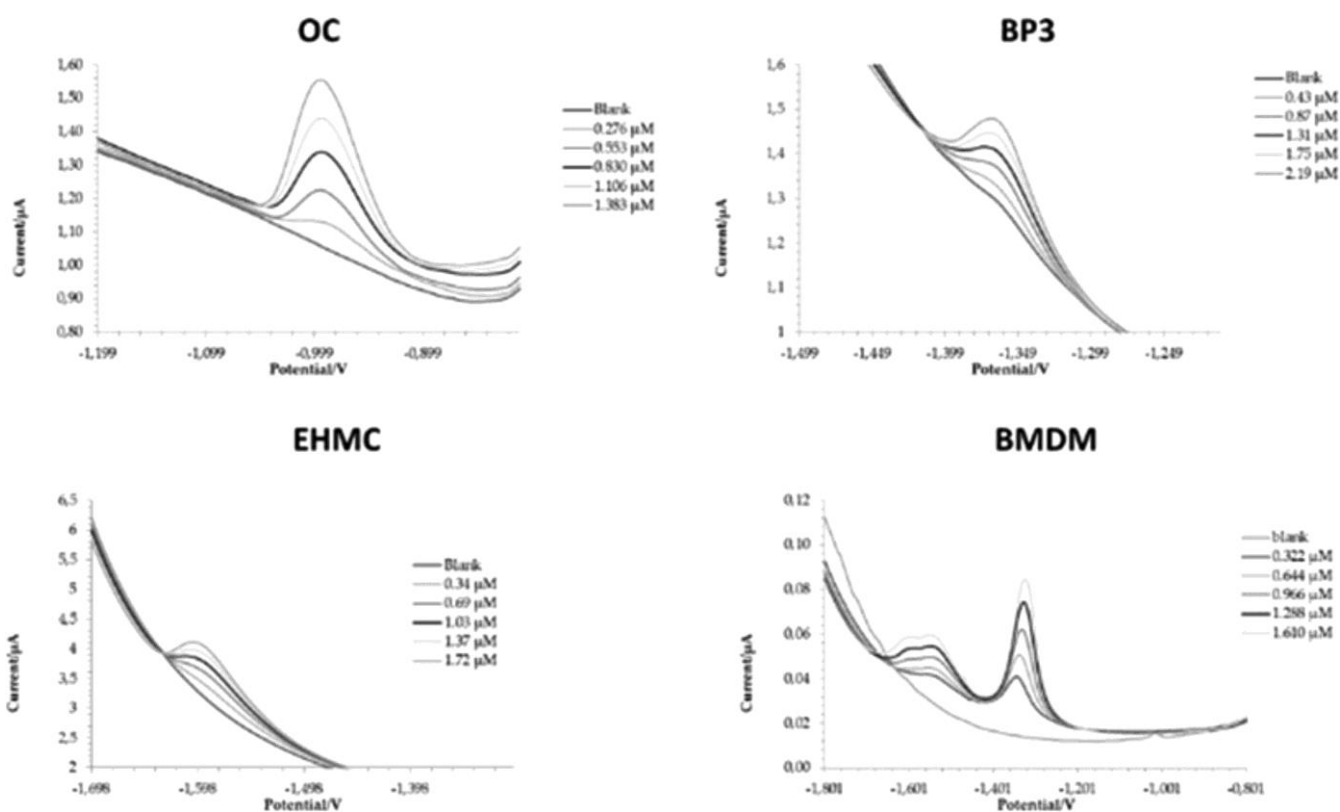
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173 **Table 2. SWV parameters (E_{dep} = Deposition potential, t_{dep} = Deposition time, t_{eq} = Equilibration**
174 **time, E_{start} = Start potential, E_{end} = End potential, E_{step} = Step of potential, A = Amplitude, and F =**
175 **Frequency)**

parameter	value
E_{dep}	-0.4 V
t_{dep}	60 s
t_{eq}	20 s
E_{start}	-0.8 V
E_{end}	-1.7 V
E_{step}	0.009 V
A	0.021 V
F	50 Hz

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179 Using these parameters set and as a solution consisting of 8.0 mL of 0.1 M NaCl, 1.0 mL of
180 methanol, 1.0 mL of ethanol, and 0.30 mM CTAB as support electrolytes, the quantifications on the
181 single analytes were evaluated. SI Figure S3 reports the calibration curves for the single titled
182 analytes obtained after five additions in a solution of 0.1 M NaCl, EtOH, MeOH, and 0.30 mM CTAB.
183 In Figure 1, OC: octocrylene, BP3: benzophenone-3, EHMC: ethylhexyl methoxycinnamate, and
184 BMDM: butyl methoxydibenzoylmethane were reported for the voltammograms obtained during
185 the analyses of the single UVFs subjected to the standard addition method, while Table 3 shows the
186 performance of the method for their determination in the solution, highlighting the excellent results
187 in terms of linearity, accuracy, repeatability, and sensitivity achieved.
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Figure 1. Voltammograms for the titled analytes obtained after five additions in a solution of 0.1 M NaCl, EtOH, MeOH, and 0.30 mM CTAB.

Table 3. Performance obtained by the method in a single UVF chemical standard solution consisting of 0.1 M NaCl, MeOH, EtOH, and 0.30 mM CTAB

parameters	OC ^a	BP3 ^a	EHMC ^a	BMDM ^a
concentration range (μM)	0.276–1.38	0.483–2.19	0.34–1.72	0.322–1.61
correlation coefficient (R^2)	0.998	0.997	0.994	0.997
sensitivity ($\mu\text{A}/\mu\text{M}$)	0.35	0.058	0.55	0.28
recovery (%)	93.2	96.7	93.2	97.0
RSD (%)	3.62	3.22	0.67	3.19
LOD (μM)	0.07	0.11	0.065	0.05
LOQ (μM)	0.25	0.39	0.223	0.17

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^aFive additions of additions of: OC (0.276 μM); BP3 (0.483 μM); EHMC (0.34 μM); BMDM (0.322 μM).

Simultaneous Determination of OC, BP3, and EHMC in Solution

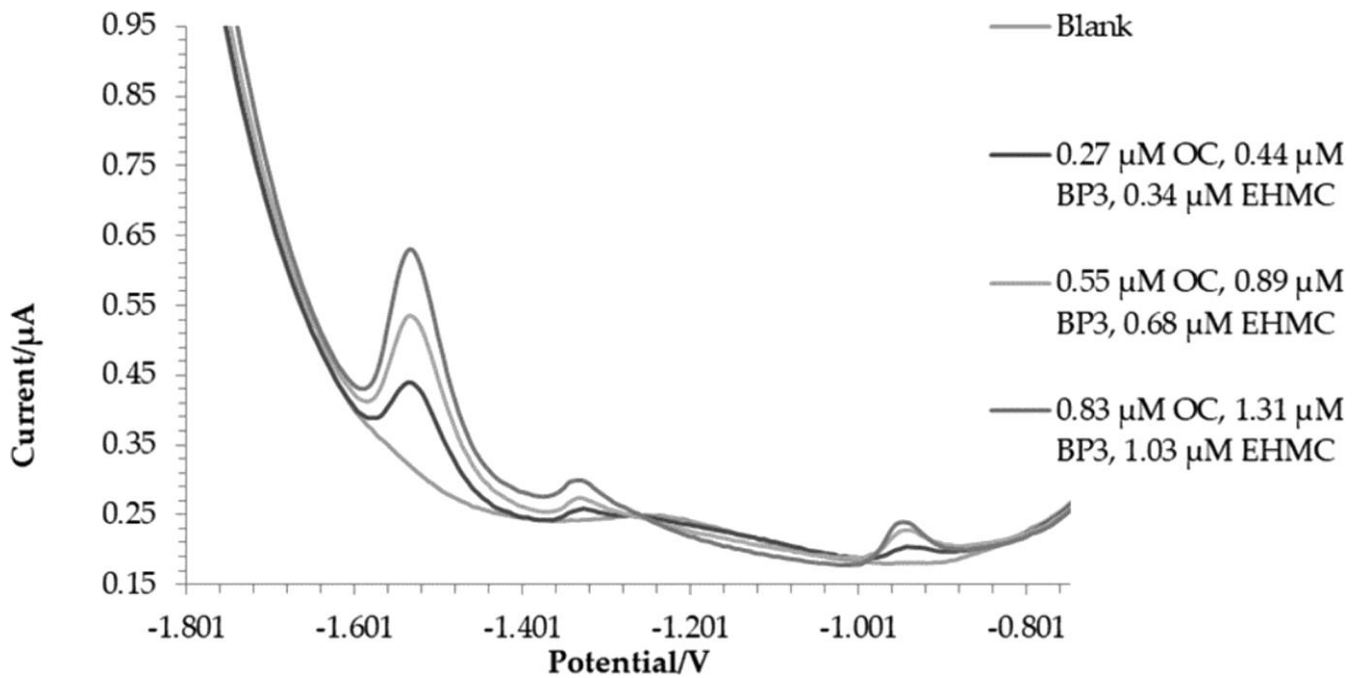
Given the satisfactory results obtained for individual analytes using the supporting electrolyte consisting of NaCl, ethanol, methanol, and 0.30 mM CTAB, it was decided to use the same solution and simultaneously add the three analytes. Three additions were made for OC, each at 0.69 μM , three additions for BP3, each at 1.09 μM , and three additions for EHMC, each at 0.86 μM . The optimized parameters shown in Table 2 were adopted for the voltammetric measurement. The corresponding voltammograms are displayed in Figure 2.

Using all of the previously optimized conditions, good results were obtained in terms of linearity, repeatability, and accuracy for all analytes simultaneously present in the solution. The performances obtained for the simultaneous determination of OC, BP3, and EHMC are reported in Table 4.

For BMDM, it was not possible to perform a simultaneous analysis with the other analytes, as it has a reduction potential of -1.4 V , equal to that of BP3. In addition, the BMDM has a second peak that falls to -1.6 V , the region of potential where the EHMC reduction signal is present. It can be observed that the simultaneous presence of the three analytes (OC, BP3, and EHMC) does not seem to affect quantification, as the results obtained exhibit high accuracy and precision. When compared with the method developed by Sunyer et al.,¹⁹ the proposed methodology appears to be more sensitive and has lower LOD and LOQ values. Compared to the method developed by Ferreira et al.,²⁰ the proposed methodology has higher LOD and LOQ values for OC. The developed method is not only more sensitive for EHMC detection but even more environmentally friendly, in comparison with Ferreira and Cardoso et al., since they use a hanging mercury drop electrode^{2,19}.

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225 Figure 2. Voltammograms obtained following three additions of OC (0.27 μM), BP3 (0.44 μM), and
 226 EHMC (0.34 μM) in a solution of 0.1 M NaCl, EtOH, MeOH, and 0.30 mM CTAB.

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228

229 **Table 4. Performance Obtained by the Method Following Three Additions of OC (0.27μM), BP3**

230 **(0.44 μM), and EHMC (0.34 μM) in a Solution Consisting of 0.1 M NaCl, MeOH, EtOH, and 0.30 mM**
 231 **CTAB**

parameters	OC	BP3	EHMC
concentration range (μM)	0.27–0.83	0.44–1.31	0.34–1.03
correlation coefficient (R^2)	0.995	0.994	0.995
sensitivity (μA/μM)	0.16	0.08	0.42
recovery (%)	91.7	96.7	100.0
RSD (%)	1.96	0.93	3.48
LOD (μM)	0.06	0.09	0.08
LOQ (μM)	0.21	0.29	0.26

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233 **Analysis on Cosmetic Samples**

234 ***Optimization of the Extraction of Analytes from Cosmetics***

235 Several extraction procedures were tested to extract analytes from the sunscreens. In order to
236 obtain an exhaustive extraction of the different analytes, the type of solvent, the amount of solvent,
237 and the type of mechanical pretreatment (ultrasound and mechanical agitation) have been tested.

238 All extraction procedures were tested on a sunscreen sample (reported in Table S9 in the SI).

239 The procedure for exhaustively extracting analytes is as follows: (i) the sunscreen is carefully
240 weighed (0.1 g) and placed in 50 mL of plastic test tube; (ii) 50 mL of 0.1 M NaCl is added; (iii) the
241 solution is kept under stirring for 30 min; (iv) 0.1 mL of the solution is placed in an electrochemical
242 cell with 8 mL of 0.1 M NaCl, 1.0 mL of methanol, 1.0 mL of ethanol, and 0.30 mM CTAB; and (v)
243 quantification is performed by the method of standard additions. Finally, the percentage (% w/w) of
244 UV filters present in each sunscreen sample was calculated.

245 The results obtained by voltammetry were compared with those obtained through HPLC-PDA and
246 HPLC-MS/MS analyses, if the latter provides accurate results.

247 For both the HPLC-PDA and HPLC-MS/MS analyses, 0.1 g of sunscreen was added to 4 mL of
248 ethanol, and the solution was sonicated for 15 min. To the sample, 6 mL of mobile phase, composed
249 of HPW and acetonitrile acidified with 0.1% of formic acid (52:48 v/v), was added, and the mixture
250 was centrifuged for 10 min (4000g). Subsequently, the sample was diluted 1:200 with the mobile
251 phase, and after filtration through a 0.45 mm PTFE syringe filter, 5 μ L of the solution was injected.

252 In the SI, various previous tests were reported, first to obtain the optimized extraction procedure.

253 It was observed that the extraction procedure yielding the best results in terms of % w/w is
254 extraction procedure A. This procedure provides percentages that closely approximate those
255 obtained with HPLC-PDA and HPLC-MS/MS. After optimizing the sample pretreatment, various
256 cosmetics were analyzed. The results are reported in Table 5 compared to those obtained with
257 other instruments. Some voltammograms of the analysis of the samples are reported in the SI,
258 Figure S4.

259 For all of the analyzed cosmetic samples, the results obtained by means of the voltammetric
260 technique were in good agreement with those obtained using a conventional method, such as
261 HPLC-PDA and HPLC-MS/MS. Therefore, the optimized analytical procedure proved to be suitable
262 for determining UV filters in complex matrices such as sunscreens or lipsticks.

Table 5. Quantification of UV filters in cosmetics—Results reported as % (w/w) of UVFs, voltammetry vs HPLC-PDA and HPLC-MS/MS

sample	HPLC-PDA			HPLC-MS/MS			voltammetry		
	EHMC	OC	BMDM	EHMC	OC	BMDM	EHMC	OC	BMDM
1	3.49 ± 0.3			2.17 ± 0.20			3.54 ± 0.4		
2	2.48 ± 0.3	2.30 ± 0.2	0.14 ± 0.02	2.06 ± 0.17	1.80 ± 0.14	0.09 ± 0.01	2.47 ± 0.24	2.31 ± 0.08	
3		3.64 ± 0.4	1.51 ± 0.2		2.01 ± 0.21	1.10 ± 0.12		3.44 ± 0.21	1.51 ± 0.11
4		2.80 ± 0.3	1.85 ± 0.2		2.49 ± 0.29	1.01 ± 0.11			
5			3.81 ± 0.4			1.95 ± 0.23			3.81 ± 0.07
6			2.19 ± 0.2			1.53 ± 0.17			2.04 ± 0.03
7			1.26 ± 0.1			1.09 ± 0.12			1.53 ± 0.04
8	1.38 ± 0.1	2.46 ± 0.2	1.73 ± 0.2	0.80 ± 0.09	1.99 ± 0.22	1.97 ± 0.23	1.25 ± 0.08	2.42 ± 0.21	1.75 ± 0.1
9			0.17 ± 0.02			0.03 ± 0.01			<LOQ
10	2.32 ± 0.2			2.00 ± 0.22			2.09 ± 0.12		
11	0.03 ± 0.01		4.28 ± 0.4	0.18 ± 0.03		3.87 ± 0.39	<LOQ		4.42 ± 0.23
12	1.13 ± 0.1	0.26 ± 0.03	1.03 ± 0.1	1.87 ± 0.21	0.22 ± 0.03	1.32 ± 0.16	1.3 ± 0.2	<LOQ	
13	4.14 ± 0.4		0.74 ± 0.1	4.52 ± 0.38		0.99 ± 0.12	4.22 ± 0.54		<LOQ
14		1.17 ± 0.1	1.30 ± 0.1		1.01 ± 0.11	1.11 ± 0.13		1.3 ± 0.35	1.32 ± 0.05
15			0.26 ± 0.03			0.35 ± 0.03			<LOQ
16		5.37 ± 0.5			6.66 ± 0.54			4.11 ± 0.02	
17	3.70 ± 0.4			2.01 ± 0.18			2.68 ± 0.33		
18		8.85 ± 0.7	4.61 ± 0.4		6.64 ± 0.73	3.92 ± 0.42		8.38 ± 0.67	3.6 ± 0.48
19	1.84 ± 0.2		1.29 ± 0.1	1.88 ± 0.21		1.29 ± 0.13	2.1 ± 0.19		1.17 ± 0.28

266 In terms of the percentage of filters found in the products, all results comply with the limits set by
 267 the European Chemicals Agency (ECHA) (OC < 10%, EHMC < 10%, BMDM < 5%). In fact, all analyzed
 268 sunscreen samples exhibit UV filter percentages much lower than the legal limits. Therefore, these
 269 sunscreens are considered “safe” from an environmental standpoint, although continued use could
 270 potentially have various impacts on the aquatic ecosystem.

271

272 **Table 6. Pearson correlation matrix**

variable	EHMC PDA	EHMC MSMS	EHMC ASV
EHMC PDA	1	0.716	0.902
EHMC MSMS	0.716	1	0.916
EHMC ASV	0.902	0.916	1
	OC PDA	OC MSMS	OC ASV
OC PDA	1	0.928	0.990
OC MSMS	0.928	1	0.876
OC ASV	0.990	0.876	1
	BMDM PDA	BMDM MSMS	BMDM ASV
BMDM PDA	1	0.887	0.966
BMDM MSMS	0.887	1	0.816
BMDM ASV	0.966	0.816	1

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CONCLUSIONS

In this work, the possibility of using electrochemical techniques for the determination of UVFs contained in sunscreens was demonstrated and validated. In particular, the whole method has been optimized for the determination of OC, BP3, EHMC, and BMDM; the results, reported as a percentage w/w, have been compared with those obtained by means of originally "ad hoc" developed and validated methods based on HPLC-PDA and HPLC-MS/MS.

An interesting element is represented by the fact that in the present work not only are the SWV method and a portable device developed and validated but also everything is compared with two reference configurations (HPLC-PDA and HPLC-MS/MS). In this case, the chromatographic method has demonstrated the necessary ruggedness to be applied directly to two different instrumentations, obtaining comparable performance and without any method transfer problem. The electrochemical method developed herein allows for carrying out reliable analyses potentially everywhere, as it uses completely portable instrumentation. This represents a great innovation to increase the number of controls on different matrices by using a low-cost, portable, easy-to-use, and green technology.

One of the main objectives of green analytical chemistry (GAC) and green sample preparation (GSP) lies in the possibility of carrying out in situ measurements using simple instrumentation and with the use of nontoxic reagents and solvents, reducing sample manipulation and the number of steps related to pretreatment. The method and portable device presented here allow us to be compliant with these principles, highlighting how this approach can be considered green and low impact, paving the way for its new applications also in other fields (i.e., environmental and biological ones), in order to monitor the presence of these potential CECs in different settings.

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