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HPLC method with electrochemical detection on gold electrode for simultaneous determination of different antimicrobial agents in cosmetics

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ABSTRACT

Antimicrobial agents are added to a wide variety of products such as cosmetics to reduce the risk of microbial contamination and ensure the suitability and safety of the product. However, recent studies have shown that these compounds can have negative effects on human health and the environment. Therefore, it is crucial to develop analytical methods to control the amount of these compounds in personal care products to guarantee human health and product quality. This paper presents an HPLC method with electrochemical detection using a gold electrode for the determination of the most used antimicrobial agents in cosmetic products: methylparaben (MP), 4-hydroxybenzoic acid (4-HBA), phenoxyethanol (PE) and methylisothiazolinone (MIT). For this purpose, the electrochemical response of these compounds was evaluated on the gold electrode. The optimal electrochemical detection of MP, 4-HBA; PE and MIT was performed at pH 2 and + 1.50 V (*vs* Ag/AgCl). Under the optimal separation and detection conditions, limits of detection (LODs) between 10 and 110 μg L^{-1} were obtained. These LODs are lower than those previously reported for other HPLC methods with mass spectrometry and diode-array detectors. Cosmetic products with different compositions were successfully analysed with the proposed HPLC method obtaining recoveries between 76 % and 119 %.

1. Introduction

Antimicrobial agents or biocides are defined in the Regulation (EU) No 528/2012 [1] as "any substance or mixture used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action". Biocides can be classified into four main groups: disinfectants such as ethanol or benzoic acid, preservatives (e.g., parabens or isothiazolinones), pest control such as paraquat or atrazine, and other biocidal products such as glycerol or formaldehyde. Biocides can be incorporated into a wide variety of products that are daily used by the society, (e. g. products for human and veterinary hygiene, drinking water, food packaging, cosmetics, or medicinal products).

Cosmetics have become essential products for people's daily lives, contributing to well-being and healthy lifestyle [2]. In this way, these products are one of the main sources of human exposure to antimicrobial agents. Alcohol-type and the isothiazolinones are among the most used compounds to prevent microbial and algae growth in cosmetic products [3]. Mixtures of these types of antimicrobial agents are usually added to cosmetics to enhance the antimicrobial activity. Alcohol-type preservatives are characterized by the presence of hydroxyl groups in their structures and having acid-base properties. Notable of this group are phenoxyethanol (PE) and parabens. PE is an aromatic alcohol and ether with a broad spectrum but weak antimicrobial activity against different Gram-positive and Gram-negative bacteria and it is usually added in combination with other antimicrobial agents in cosmetics [4]. Parabens are widely used for cosmetic preservation due to their excellent properties, e.g., good stability, effective in a broad pH range and active against Gram-positive bacteria and fungi [5]. These compounds are esters of 4-hydroxybenzoic acid (4-HBA) and their properties depend on the length of the alkyl chain. Antimicrobial activity increases with the length of the alkyl chain, however a decrease of water solubility is also observed. For this reason, methylparaben (MP) is the most used, alone or in combination with other antimicrobial agents, for the preservation of cosmetics [6,7]. Although the stability of parabens in cosmetic formulations is high, the hydrolysis to 4-HBA can occur, affecting antimicrobial activity and thus consumer safety [8]. Isothiazolinones are five-membered heterocyclic compounds that contain a nitrogen-sulphur bond responsible of the high activity, even at low concentrations,

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against bacteria, microbes, virus, and fungi. Among isothiazolinones, methylisothiazolinone (MIT) is the most widely used [9].

Considering the extensive use of personal care products, the concern about the risks of cosmetic's ingredients in human health is increasing. It is well known that parabens are endocrine disruptors and recent investigations have detected their presence in tumours and tissues [10,11]. Not only parabens but also PE and MIT have negative effects on human health, being antimicrobial agents along with metals and fragrances the main cause of allergic contact dermatitis [4,12–15]. To guarantee the safety of the consumers, the maximum concentration of biocidal products added to cosmetics is regulated by the European Union in the Regulation (EU) No 1223/2009 [16]. The maximum allowed concentration of parabens in cosmetic products is 0.4 % for a single ester and 0.8 % for mixtures of parabens expressed as 4-HBA [16,17]. The maximum permissible concentration of PE in personal care products is established in 1.0 % [16]. The use of MIT in *leave-on* products is banned since 2017, being 0.0015 % the maximum concentration that can be found in personal care products [18].

The development of analytical methods for the determination of antimicrobial agents from different families in personal care products is required to guarantee consumer safety and compliance with the regulation. Cosmetics are formed by mixtures of different compounds so separation techniques such as gas chromatography [19,20], capillary electrophoresis [21,22], and especially liquid chromatography [23] are the most used to determine antimicrobial agents. Most of the published methods are focused on the determination of parabens [23], being scarce the methods for the simultaneous determination of preservatives from different families [24–26]. Diode-array (DA) and UV coupled to HPLC and UHPLC are the most used detectors for the identification of antimicrobial agents [23–29]. However, due to the complexity of cosmetic matrices, more selective detectors (e.g., mass spectrometer (MS), fluorescence (FLD) and electrochemical detector (ECD)) are needed. Methods based on HPLC-MS and HPLC-MS/MS has been developed for the determination of parabens [30], PE [31], and MIT [32]. FL and EC detectors have been also used for the quantification of parabens achieving limits of detection at the μg per litre level [33,34]. However, no HPLC methods coupled to ECD have been developed for the determination of PE and MIT, even though in recent years electroanalytical methods for the determination of PE [35] and MIT [36] on platinum and gold electrodes, respectively, have been published, demonstrating the possibility of using ECD for their determination. Due to the wide variety and complexity of cosmetic matrices, sample pretreatments are required to perform the analysis. Depending on cosmetics characteristics, sample pre-treatment can vary from a simple dilution [22] to solid-phase extraction [37], ultrasound assisted extraction [38], liquid–liquid microextraction [39], or pressurized liquid extraction [19].

In this work we have implemented the electrochemical detection using a gold working electrode for the HPLC determination of MP, MIT, and PE in cosmetic products. To obtain information about the hydrolysis of parabens in the samples, 4-HBA has been also analysed in this work. Gold was selected as working electrode to achieve a simultaneous determination of all antimicrobial agents, since as has been demonstrated in previous works, MIT is not electroactive on carbon-based electrodes [40]. As far as our knowledge, there are no previous HPLC-ECD-based works for the simultaneous determination of alcohol and isothiazolinone-type bioactive compounds. The good electrochemical properties of these compounds on the gold electrode allow a selective HPLC determination with low limits of detection and quantification.

2. Experimental section

2.1. Reagents

The reagents used throughout this work were all analytical grade and were used with any further purification. MP (99.8 %), 4-HBA (99 %), MIT (95 %) and PE (99 %) were purchased from Sigma-Aldrich (Saint Louise, USA). Ultrapure water (resistivity $\geq$ 18 MΩ cm) obtained from a Milli-RO-Milli-Q water system (Millipore, Massachusetts, USA) was used for the preparation of the solutions. HPLC-grade acetonitrile and methanol were acquired from Scharlab (Barcelona, Spain). Stock solutions of the analytes at a concentration of 1000 mg L^{-1} were prepared in a 50 % (v/v) water: acetonitrile mixture and stored under refrigeration in the darkness. Standard solutions for the HPLC analysis were obtained by diluting the adequate volume of each compound stock solution in the mobile phase (5 % acetonitrile: 95 % 0.050 mol/L phosphate buffer solution at pH 2).

Ortho-phosphoric acid (85 % w/w) and sodium and potassium phosphate salts used to adjust the pH of the solutions were from Merck (Darmstadt, Germany) and Fluka (Saint Galen, Switzerland), respectively. Sodium hydroxide (Scharlab, Barcelona, Spain) were used to adjust the pH of the mobile phase.

2.2. Instrumentation

Electrochemical measurements were performed using the Autolab PGSTAT302N Potenciostat/ Galvanostat (EcoChemie). The General-Purpose Electrochemical System (GPES 4.9007 version) software was used for data acquisition and handling. The electrochemical cell consisted of a gold disk electrode (2 mm of diameter, model CHI101, CH Instruments, USA) as working electrode, a Ag/AgCl (3 mol/L KCl, Metrohm, Spain) as reference electrode and a platinum ring as counter electrode.

The separation of the four antimicrobial agents was carried out on a Jasco™ HPLC system (Tokyo, Japan) formed by a quaternary pump (Jasco PU-2089 plus, Tokyo, Japan) equipped with a vacuum degasser, an automatic sample injector (Jasco AS-2055 plus, Tokyo, Japan), an oven for the chromatographic column (Jasco CO-2067 plus, Tokyo, Japan) and a diode-array detector (Jasco MD-2010 plus, Tokyo, Japan) equipped with 1024 photodiodes. A LC-Net II/ ADC interface (Jasco, Tokyo, Japan) and the Jasco ChromPass Software were used for data handling and acquisition, and to control the HPLC system.

Hydrodynamic voltammograms were recorded using a flow injection analysis (FIA) system formed by a peristaltic pump (model 302C, Gilson, Middleton, USA), and an injection valve (Omnifit, 21939-18) equipped with a 100 μL sample loop.

A *wall-jet* electrochemical cell (model 656, Metrohm) formed by a gold working electrode (3 mm of diameter, Metrohm), a Ag/AgCl in 3.0 mol/L KCl reference electrode (6.0727.000, Metrohm) and a gold counter electrode was used for the electrochemical detection in the HPLC and FIA systems. The Autolab PGSTAT302N Potenciostat/ Galvanostat (EcoChemie) controlled by GPES software was used to record the amperometric signals and control the applied working potential.

2.3. Electrochemical measurements

The electrochemical behaviour of the target analytes at 200 mg L^{-1} concentration level on the gold electrode was studied by cyclic voltammetry in 0.10 mol L^{-1} phosphate solutions at different pH values in the working pH range of the chromatographic column (between 2 and 8) in the presence of 10 % of acetonitrile. Cyclic voltammograms were recorded in a potential range from −0.25 V to + 1.70 V at a scan rate of 0.100 V s^{-1} and 4 mV of step potential. Between measurements, 30 successive scans in 0.50 mol L^{-1} KOH (Sigma-Aldrich, Saint Louise, USA) at 0.100 V s^{-1} were performed to remove any product adsorbed on the electrode surface and ensure the reproducibility of the voltammetric measurements.

Hydrodynamic voltammograms of MP, HBA, MIT and PE at a concentration of 10.0 mg L^{-1} were obtained in the FIA system using a mixture 20:80 of acetonitrile: 0.050 mol L^{-1} phosphate buffer at pH 2 at 4.0 mL min^{-1} as carrier.

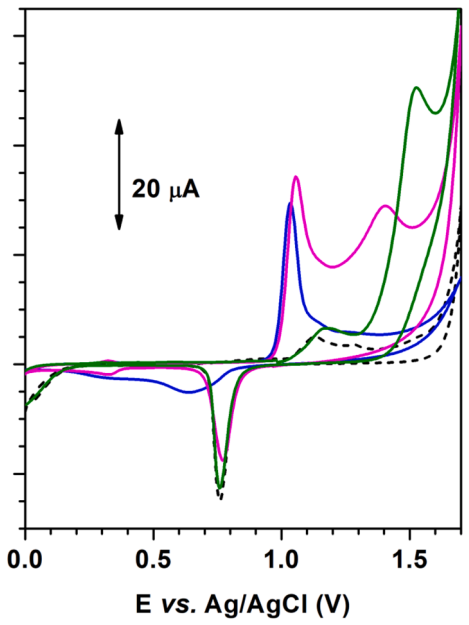

Fig. 1. Cyclic voltammograms for 200 mg L^{-1} concentration solutions of MP (—) 4-HBA (—), and PE (—) on the gold electrode. Conditions: 10 % Acetonitrile: 90 % 0.10 mol L^{-1} phosphate buffer at pH 2 (– – –), 0.100 V s^{-1} scan rate.

2.4. Chromatographic and detection conditions

Separation of MIT, 4-HBA, PE and MP was carried out on a Kromasil C18 HPLC column (150 mm length × 4.6 mm of inner diameter, 5 μm particle size, Scharlab Barcelona, Spain) at 40 °C and using 20 μL as injection volume. The mobile phase was composed by acetonitrile and 0.050 mol/ L^{-1} phosphate buffer at pH 2 in a gradient mode and using a flow rate of 1.0 mL min^{-1}. First a linear gradient from 5 % to 10 % of acetonitrile in 5 min was performed. Then the acetonitrile percentage was increased up to 25 % in 2 min and this percentage was maintained for 8 min. After that, the initial conditions (5 % of acetonitrile) were restored in 1 min and this percentage was maintained for 2 min [41]. The acetonitrile percentage was increased from 25 % to 100 % prior to return to the initial conditions when samples were analysed. The electrochemical detection was carried out in amperometric mode applying + 1.50 V *vs.* Ag/AgCl as working potential. To guarantee the reproducibility of the measurements, a pretreatment of the working electrode was carried out in the HPLC system before each injection. This pretreatment consisted in 10 cyclic voltammograms performed between −0.50 V and + 1.50 V at 0.100 V s^{-1} previous the application of the working potential.

2.5. Sample preparation

Four cosmetic samples with different characteristics in texture and composition were analysed; specifically: a facial tonic, a shampoo, a hair conditioner and, a body cream containing at least one of the studied antimicrobial agents. These cosmetics were manufactured between 2017 and 2019 and acquired from commercial supermarkets.

Considering the complexity in the composition of cosmetic products, different sample treatments were used [41]. Liquid aqueous samples, such as the facial tonic only required a dilution with ultrapure water prior the analysis. Antimicrobial agents were extracted from cosmetics containing surfactants, waxes and/or oil-based using the following ultrasound assisted extraction (UAE) procedure: 10.0 mL of methanol were added to 0.5 g of the sample and the mixture was sonicated in the ultrasonic bath (model P30HS Elmasonic, Singen, Germany) for 10 min at room temperature, at a frequency of 80 kHz and 100 % of power. The extract was obtained after 5 min of centrifugation at 2500 rpm. Prior the analysis, all sample solutions were filtered through a 0.45 μm nylon syringe filter.

3. Results and discussion

3.1. Electrochemical behaviour of antimicrobial agents on gold electrode

First, the electroactivity of the antimicrobial agents on gold electrode was study by cyclic voltammetry. The electrochemical behaviour of MIT on gold electrode was previously evaluated in our laboratory [36]. In short, this compound is electroactive on gold electrode in the pH range from 2 to 10. Two anodic peaks were observed in the voltammograms of MIT at pH values lower than 5 and its oxidation occurs at potentials higher than the anodic potential of gold. In addition, a displacement of the anodic peak of gold to more positive potentials was observed in the presence of MIT, indicating the adsorption of the compound on the electrode surface. No significant changes on the cathodic peak of gold were observed in the presence of the isothiazolinone [36]. To evaluate the electrochemical response of MP, 4-HBA, and PE on gold electrode, the cyclic voltammograms of solutions of these compounds at 200 mg L^{-1} in the presence of 10 % of acetonitrile were registered. Fig. 1 shows the cyclic voltammograms obtained and as can be seen in the absence of analytes two anodic peaks and a cathodic peak related to the formation of different gold oxides and its reduction, respectively, are observed. The voltammogram for MP presents an anodic peak at + 1.04 V so the

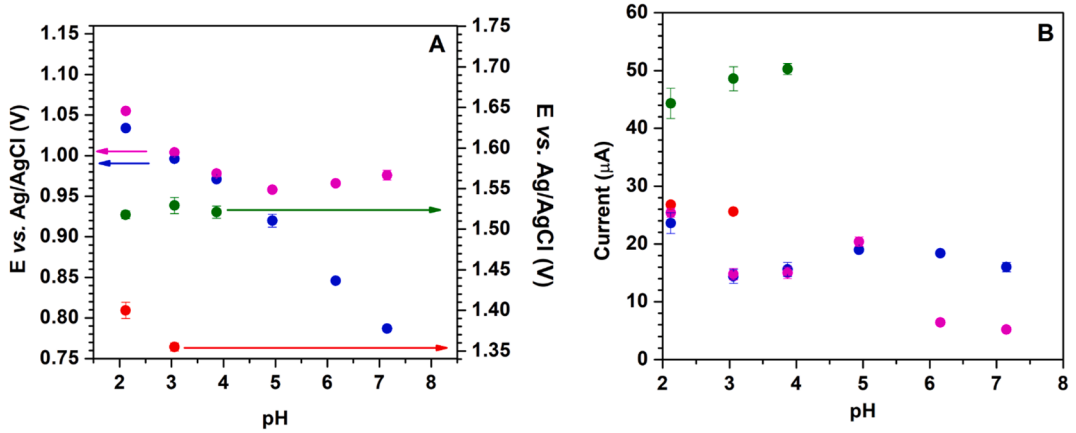

Fig. 2. Effect of pH on (**A**) the anodic peak potential and (**B**) peak currents of MP (●),4-HBA anodic peaks (the first one (●) and second one (●), and PE (●) at a concentration of 200 mg L^{-1}. Mean values ± standard deviation of 3 replicates.

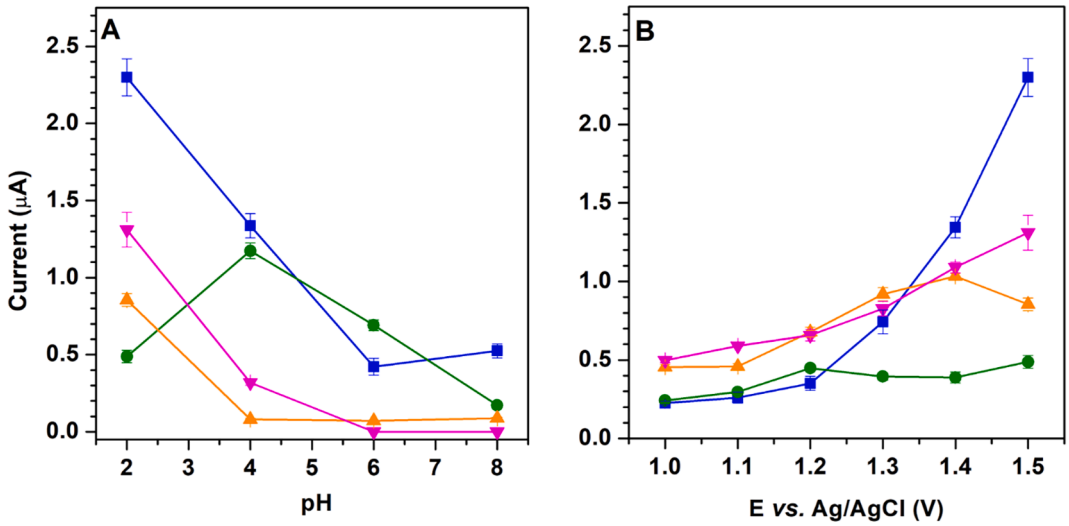

Fig. 3. Optimization of the electrochemical detection on the gold electrode of MP (■), 4-HBA (▼), MIT (▲), and PE (●) at 10.0 mg L^{-1}concentration level: (**A**) Effect of pH on anodic peak current using 0.050 mol L^{-1} phosphate solution containing 20 % of acetonitrile as carrier and applying + 1.50 V (*vs* Ag/AgCl). (**B**) Effect of applied potential on electrochemical response using 0.050 mol L^{-1} phosphate buffer at pH 2 containing 20 % of acetonitrile as carrier.

oxidation of this compound occurs prior the formation of gold oxides [42]. In the presence of the paraben, the cathodic peak of gold shifts to less positive potential values and the peak current diminishes. This behaviour indicates that MP blocks the surface oxidation of gold. These results are similar to those previously published [43]. Two anodic peaks at + 1.04 V and + 1.40 V are observed in the voltammogram of 4-HBA. In the presence of the analyte, a diminution of the current of the cathodic peak of gold at + 0.76 V is observed indicating the interaction of 4-HBA with gold oxides. An anodic peak at + 1.50 V is observed in the voltammograms of PE and no variations are observed on the cathodic peak. The anodic peak potential value for PE is similar to that observed in previous work [35].

The electrochemical response of the analytes was studied at different pH values. In our previous work, MIT response was also evaluated at different pH, and a linear dependence of anodic peak potential with the pH was observed [36]. According to the slope value, the same number of electrons and protons are exchanged during the oxidation process. MIT oxidation on gold electrode is a diffusion-controlled process in which two electrons and two protons are exchanged to obtain the sulfoxides and finally the sulfones [36]. Fig. 2A shows the effect of pH on the anodic peak potentials of MP, 4-HBA and PE. The anodic peak potentials of MP and 4-HBA linearly decreases towards lower values as pH increases, indicating that protons are released in the electro-oxidative process. For the first anodic peak of 4-HBA (pink circles in Fig. 2A), peak potential becomes pH-independent at pH values higher than 5 due to the deprotonation of this compound (pK_a 4.57). The potential of the second anodic peak of 4-HBA (red circles in Fig. 2A) was also pH dependent. The peak potential values for MP and 4-HBA linearly decreased with a slope of about −60 mV per pH unit, suggesting that the same number of electrons and protons are exchange in the oxidation process. Similar results were obtained by other authors [43,44]. According to previous works, the oxidation of MP and 4-HBA is a diffusion-controlled process in which an electron and a proton are exchanged [43,44]. For MP, the phenolic group is oxidized to form the phenoxyl radical [43] and in the case of 4-HBA during the oxidation process a hydroxyl group can be incorporated to the structure in *para*- and *ortho*-positions [44]. As can be seen in Fig. 2A, the anodic peak potential of PE can be considered independent of the pH value. M. Jakubcyzk *et al.* proposed in their work that the oxidation of PE is a diffusion-controlled process in which one electron is transferred, and as in this work, no exchange of protons occurred during the anodic oxidation of PE [45]. The oxidation of PE proceeds on the etheric oxygen atom. Cation radicals are obtained, and they react to form different final products such as ethylene glycol and dimers [45].

The influence of pH solution value on the anodic peak current of MP, 4-HBA and PE is presented in Fig. 2B. The maximum anodic current for MP and 4-HBA is observed at pH 2. For PE, the peak current increases as the pH value increases, achieving the maximum at pH 4. The previous study on the electrochemical behaviour of MIT shown the variation of the anodic peak current with the pH values [36].

3.2. Optimization of the electrochemical detection

To select the most adequate conditions for the electrochemical detection of MIT, 4-HBA, PE and MP on the gold electrode, the influence of the pH of buffer solution and the applied potential on peak current was evaluated. For this purpose, 10.0 mg L^{-1} standard solutions of MP, 4-HBA, PE and MIT were injected by triplicate in the FIA system using 0.050 mol L^{-1} phosphate solution containing 20 % of acetonitrile as carrier. Fig. 3A shows the effect of pH on the electrochemical response of the target analytes applying + 1.50 V as working potential. As expected, the results for PE, MP and 4-HBA were similar to those obtained using cyclic voltammetry, achieving the maximum peak current at pH 4 for PE and at pH 2 for MP and 4-HBA. As can be seen in Fig. 3A, MIT presents a similar behaviour than 4-HBA, observing the maximum of the anodic current at pH 2 and very low signals when the pH is higher than 4. Fig. 3B shows the hydrodynamic voltammograms obtained using 0.050 mol L^{-1}phosphate buffer at pH 2 as electrolyte. As can be seen, for MP and 4-HBA an increase of peak current is observed with the applied potential. For MIT, the anodic current increases when the potential varies between + 1.10 V and + 1.40 V and a diminution of the signal is observed at + 1.50 V. An increase of the anodic current of PE as the applied potential increases is observed up to a potential of + 1.20 V. Similar anodic currents are observed between + 1.20 V and + 1.50 V. According to these results, to determine all compounds simultaneously with good sensitivity, pH 2 and + 1.50 V were selected as the optimal pH value and detection potential, respectively.

3.3. Optimal chromatographic and detection conditions

Optimal separation of MIT, 4-HBA, PE and MP was carried out using a mobile phase composed by acetonitrile and 0.050 mol L^{-1} phosphate buffer at pH 2 in a gradient mode and using a flow rate of 1.0 mL min^{-1}. The gradient program was previously optimized in our laboratory [41].

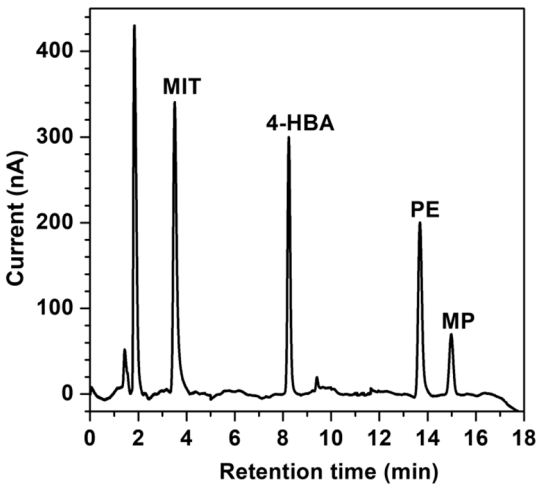

Fig. 4. Chromatogram of MIT, 4-HBA, PE and MP at 5.00 mg L^{-1} concentration level, obtained under the optimal separation and detection conditions.

In short, two linear gradients were performed from 5 % to 10 % of acetonitrile in 5 min and from 10 % to 25 % in 2 min, then this percentage was maintained in isocratic mode for 8 min. Before the next injection, the initial conditions (5 % of acetonitrile) were introduced in 1 min and this percentage was maintained for 2 min. When cosmetic samples were analysed, to avoid clogging or deterioration processes of the HPLC system due to the co-extracted compounds, a column washing is carried out increasing the acetonitrile percentage from 25 % to 100 % prior to return to the initial conditions. In the previous work, a mixture of acetonitrile and 0.050 mol L^{-1} acetate buffer at pH 4 was used as mobile phase [41], but in this work pH 2 is required to optimal detection, and therefore the influence of the pH of the mobile phase on the chromatographic separation was evaluated. For this purpose, multi-analyte standard solutions of 10.0 mg L^{-1} were injected using the different mobile phases and no differences in the retention times and resolutions were observed when both pH values were used. This was expected since the assayed pH values were lower than the pK_a values of the compounds with acid-base properties. Fig. 4 shows the typical chromatogram obtained on gold electrode under the optimal separation and detection conditions (+1.50 V as working potential). As can be seen, all antimicrobial agents can be detected using the gold working electrode. Under these optimal chromatographic conditions, the retention times of MIT, 4-HBA, PE and MP were 3.58 ± 0.05 min, 8.2 ± 0.1 min, 13.6 ± 0.1 min, 15.0 ± 0.1 min, respectively, so the separation was achieved in<16 min. Peak resolutions were higher than 1.5.

3.4. Calibration and analytical properties

The calibration curves for MIT, 4-HBA, PE and MP were obtained under the optimal chromatographic and detection conditions by injecting in triplicate multi-analyte standard solutions at concentrations ranging between 0.0500 and 10.0 mg L^{-1}. The principal analytical properties of the proposed HPLC-ECD method for the determination of MIT, 4-HBA, PE and MP are presented in Table 1. As can be seen, good linear relationships between the concentration and peak area are achieved in the studied concentration range with linear regression coefficients higher than 0.9977. The highest sensitivity, expressed as the slope of the calibration curve, is obtained for MIT. For all compounds, the intercept values of the calibration curves were statically equal to zero at 95 % – confidence level. Instrumental limits of detection (ILOD) and quantification (ILOQ) were calculated using a signal-to-noise ratio of 3:1 and 10:1, respectively, from the standard deviation of the peak area for a standard solution with the minimum concentration at which the analytes can be reliably detected. According to the ILOQs of the technique (Table 1) and the sample treatment procedure, the LOQs obtained for the method are between 0.0001 and 0.0007 % of preservative in the sample. Considering the maximum concentrations established in the regulations, the proposed HPLC-ECD method is adequate for the analysis of these compounds in cosmetic products. The intraday (repeatability) and interday (reproducibility) precision of the method was evaluated by injecting four replicates of 1.0 mg L^{-1} multi-analyte standard solution in the same and different days, respectively. Values of repeatability and reproducibility, expressed as relative standard deviation (%, RSD) lower than 7 % and 12 %, respectively, were obtained.

Some analytical properties, such as ILOD and %RSD, of HPLC methods used to determine MP, 4-HBA, MIT and PE are compared in Table 2 with those obtained using the proposed HPLC-ECD method. For PE and MIT, the ILODs obtained using the proposed HPLC-ECD method are lower (between 6 and 38-times) than those achieved in different HPLC-DAD methodologies [27,28,46]. Comparing with the HPLC-DAD method developed in our laboratory [40], similar ILODs are obtained for MIT, PE and 4-HBA, and an ILOD 10-times higher is obtained for MP with the proposed HPLC-ECD method. The here obtained ILOD of MIT is similar to that obtained in a UPLC-MS/MS method [32]. Comparing with other HPLC-ECD methods for the determination of MP, the ILOD obtained in this work using the gold electrode is lower than that obtained with a boron-doped diamond electrode [47] but 10-times higher than those achieved using an electrode modified with a graphene-based nanocomposite [34]. The %RSD values achieved in this work are similar to that obtained by other HPLC-ECD and HPLC-MS methods [32,34,47]. As can be seen, the published HPLC-ECD methods are

Table 2
Comparison of some analytical properties of the proposed method and other published HPLC methods for the determination of the target antimicrobial agents.

Method	ILOD (μg mL^{-1})				RSD, %	Ref.[a]
	MP	4-HBA	PE	MIT		
HPLC-DAD	0.29	–	–	0.56	<5	[27]
HPLC-DAD	0.083	–	0.33	–	<4.3	[28]
HPLC-DAD	0.018	0.029	0.066	0.011	<3.4	[41]
UPLC-DAD	0.05	–	1.55	0.175	<3.2	[46]
HPLC-MS	0.075[b]	–	–	–	<5.2	[30]
UPLC-MS/MS	–	–	–	0.033	<7	[32]
HPLC-ECD	0.4	–	–	–	<7.2	[47]
HPLC-ECD	0.01	–	–	–	<12	[34]
HPLC-ECD-Au	0.11	0.010	0.040	0.030	<12	This work

[a] Reference.
[b] Estimated ILOD taking into account the sample treatment procedure.

Table 1
Analytical properties for the determination of MIT, 4-HBA, PE and MP using the HPLC-ECD method.

Biocide	Sensitivity[a]	R^2	ILOD, μg/L	ILOQ, μg/L	Repeatability[b], %RSD	Reproducibility[b], %RSD
MIT	12.0 ± 0.2	0.9990	30	90	3.5	8.7
4-HBA	8.7 ± 0.2	0.9977	10	50	6.3	7.0
PE	6.30 ± 0.02	1.0000	40	130	2.9	8.3
MP	2.73 ± 0.05	0.9986	110	370	6.5	10

[a] Sensitivity expressed as: nA·min·L·mg^{-1}.
[b] $n = 4$.

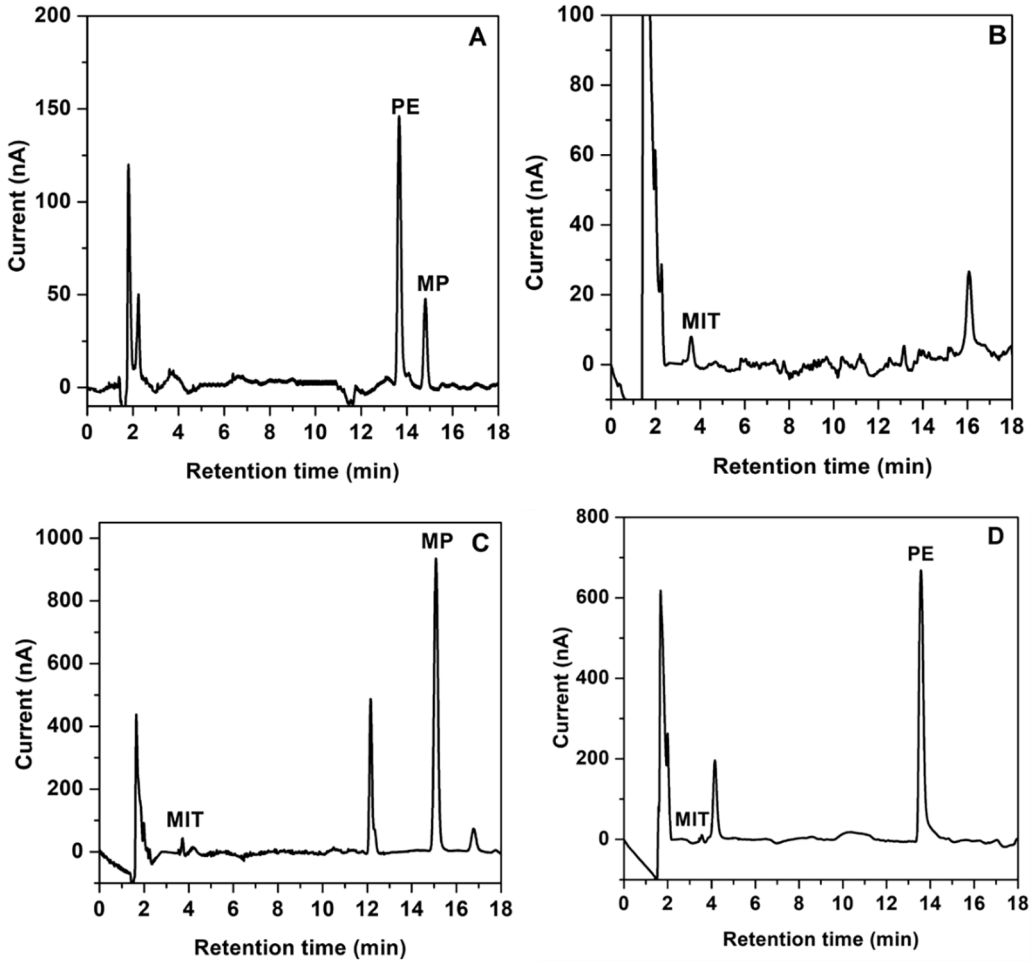

Fig. 5. Chromatograms obtained using the proposed HPLC-ECD method for (A) facial tonic (1:200 sample dilution) and extracts of (B) shampoo, (C) hair conditioner and (D) body cream.

Table 3
Concentrations of the biocides, expressed as %w/w, in the studied cosmetic samples using the here proposed HPLC-ECD method with gold electrode and a HPLC-DAD method [41].

Cosmetic sample	Declared compounds	Concentration (% w/w ± SD, $n = 3$)					
		MIT		PE		MP	
		HPLC-ECD	HPLC-DAD	HPLC-ECD	HPLC-DAD	HPLC-ECD	HPLC-DAD
Facial tonic	PE, MP			0.18 ± 0.02	0.18 ± 0.01	0.052 ± 0.004	0.059 ± 0.004
Shampoo	MIT	0.00032 ± 0.00002	0.00033 ± 0.00001				
Hair conditioner	MIT, MP	0.00011 ± 0.00002	0.00013 ± 0.00001			0.074 ± 0.008[a]	0.080 ± 0.007
Body cream	MIT, PE	0.006 ± 0.001	0.0058 ± 0.0001	0.14 ± 0.01[a]	0.169 ± 0.003		

[a] Additional 1:10 dilution.

focused on the determination of MP, whereas the proposed method allows the determination of bioactive compounds from diverse families and with different properties.

3.5. Analysis of cosmetic samples

The HPLC-ECD method with gold electrode was used for the analysis of MP, 4-HBA, PE and MIT in different cosmetic samples that according to the label contain at least one of the studied compounds. Four cosmetics with different composition and characteristics were analysed: a facial tonic, a shampoo, a hair conditioner, and a body cream. Prior the analysis, all samples were treated according to the procedure described in the Experimental Section. The analysis were performed in triplicate and using the external standard calibration method. The chromatograms of the different samples are shown in Fig. 5. PE was detected in the facial tonic and the body cream and MP was identified in the facial tonic and the hair conditioner. MIT was detected in all samples except in facial tonic.

The concentrations of MIT, PE and MP in the analysed samples are shown in Table 3. For validation purposes, the results were compared with those obtained using a previously optimized HPLC-DAD method [41]. All preservatives declared in the labels of these cosmetics were detected. The possible product of hydrolysis of parabens, 4-HBA, was not identified in any of the assayed samples, indicating the stability of parabens in cosmetic matrices. As can be seen in Table 3, in general, no significant differences (at a 95 %-confidence level) were observed between the concentrations obtained using DAD and ECD. According to the obtained results, the concentration of MIT, PE and MP do not exceed the

maximum amount established in the regulations [16–18]. Despite since 2017 the use of MIT has been banned in leave-on cosmetics, this compound was found in the body cream. This can be explained due to body cream was manufactured before 2017. Prior the amending of Regulation (EC) 1223/2003, the maximum permitted amount of MIT in leave-on and rinse-off cosmetics was 0.01 %. Thus, the amount of MIT in body cream is under the level established in the regulation.

To validate the proposed HPLC-ECD method, the samples were fortified with the antimicrobial agents and the recoveries were calculated. For this purpose, the samples were spiked with an adequate volume of the multi-analyte standard solution, and mixed properly, before applying the sample treatment. The amounts added to the samples were those to obtain fortified extracts with MIT, 4-HBA, PE and MP at concentrations between the 50 % and 100 % of the concentrations of these compounds found in the samples (Table 3). Recoveries between 76 % and 119 % with RSD values from 0.8 % to 8.3 % were obtained. These results indicate the good accuracy of the proposed method.

4. Conclusions

In this work, we propose a HPLC-ECD method to determine four of the most used antimicrobial agents in personal care products. The target compounds, methylparaben (MP), 4-hydroxybenzoic acid (4-HBA), phenoxyethanol (PE) and methylisothiazolinone (MIT) are electroactive on the gold electrode and in this way their simultaneous determination is performed in amperometric mode on the gold electrode at + 1.50 V (*vs* Ag/AgCl) detection potential. It is noteworthy that there are no previous works about an HPLC method coupled to ECD to simultaneously determine the four antimicrobial agents studied. In the works found in the bibliography, only parabens are detected using the electrochemical detector. Moreover, the method is simpler than other HPLC-ECD methods since an unmodified gold electrode is used. Under the optimal chromatographic conditions, the separation of the four antimicrobial agents is achieved in<16 min. Analytical parameters of the proposed HPLC-ECD method are comparable or better than those reported for other HPLC methods to determine these compounds. In fact, the ILODs for MIT and PE achieved with the proposed HPLC-ECD method are lower than those obtained with MS and DA detectors. Using the developed method, it is possible to determine the target antimicrobial agents in cosmetic samples with very diverse compositions, and textures, obtaining results with adequate accuracy in all cases. The LOQs of the method are lower than the maximum concentrations established in the regulations. Thus, the developed methodology is competitive and can be used to guarantee the accomplishment of cosmetics with regulations. Considering that the presence of different types of antimicrobial agents in cosmetics is common, as in the analysed products, the possibility to determine simultaneously in the same measurement run the most used antimicrobial agents is a relevant advantage of the proposed method.

CRediT authorship contribution statement

Lucía Abad-Gil: Investigation, Visualization, Writing – original draft, Writing – review & editing. **Sergio Lucas-Sánchez:** Investigation. **M. Jesús Gismera:** Supervision, Writing – original draft, Writing – review & editing. **M. Teresa Sevilla:** Writing – original draft, Writing – review & editing. **Jesús R. Procopio:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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