Article

Simultaneous Quantification of Vitamin A and Derivatives in Cosmetic Products by Liquid Chromatography with Ultraviolet Detection

Víctor Vázquez-Gomis , Sonia Carchano-Olcina, Cristian Azorín , Juan L. Benedé , Alberto Chisvert and Amparo Salvador

GICAPC Research Group, Department of Analytical Chemistry, University of Valencia, Doctor Moliner St. 50, Burjassot, 46100 Valencia, Spain; victor.vallez@uv.es (V.V.-G.); socarol@alumni.uv.es (S.C.-O.); cristian.azorin@uv.es (C.A.); Juan.L.Benede@uv.es (J.L.B.); alberto.chisvert@uv.es (A.C.)

* Correspondence: amparo.salvador@uv.es

Abstract: Vitamin A (retinol) and some of its derivatives are a group of fat-soluble compounds used in cosmetic products as bioactive ingredients. Therefore, it is necessary to perform the quality control of final product to ensure their efficacy and safety. A simple and rapid method to determine retinol, retinal, retinyl acetate, retinyl propionate and retinyl palmitate in cosmetics is presented here. The method is based on vortex and/or ultrasound-assisted leaching of the analytes in ethanol followed by liquid chromatography with ultraviolet detection. The analytical performance of the method was evaluated. It has shown high levels of linearity, at least up to $100 \mu\text{g mL}^{-1}$; high precision with RSD values below 14% and high sensitivity with low MLODs ranging between 0.3×10^{-4} and $5.9 \times 10^{-4} \% w/w$, which are enough to monitor these compounds in cosmetic products. The proposed method was successfully applied to seven commercial cosmetic samples to detect and quantify the target analytes, showing the method is suitable for its employment for quality control in cosmetic industries. Cosmetic samples were spiked at two levels of concentration and recovery values around 100% were obtained, showing no significant matrix effects and, therefore, external calibration was adequate for this determination.

Keywords: cosmetic products; fat-soluble vitamins; liquid chromatography; retinoids; vitamin A

Citation: Vázquez-Gomis, V.; Carchano-Olcina, S.; Azorín, C.; Benedé, J.L.; Chisvert, A.; Salvador, A. Simultaneous Quantification of Vitamin A and Derivatives in Cosmetic Products by Liquid Chromatography with Ultraviolet Detection. *Separations* **2022**, *9*, 40. <https://doi.org/10.3390/separations9020040>

Academic Editor: Rosa María Alonso

Received: 12 January 2022

Accepted: 29 January 2022

Published: 1 February 2022

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1. Introduction

Vitamins can be classified in two different groups as a result of their different chemical structure: water- and fat-soluble vitamins [1]. Vitamin A is classified as a fat-soluble vitamin group (i.e., retinoids) and it is used in general term to refer to retinol, retinal, retinoic acid, and retinyl esters. However, in a narrower sense, vitamin A is only referred to retinol [2]. These retinoids show structural and/or functional similarities [3], and present an essential role in human diet. Moreover, some of them, such as retinol, retinal, retinyl acetate, retinyl propionate and retinyl palmitate, have been also widely used as bioactive ingredients in cosmetic products due to their efficiency towards oxidative stress, epidermis renewing and skin diseases such as acne and psoriasis [4–6].

Retinoic acid (i.e., tretinoin) and its salts are considered as pharmaceutical drugs, present risk of teratogenicity [7] and are specifically prohibited in the European Regulation on Cosmetic Products [8]. Retinol and the other above-mentioned vitamin A derivatives are allowed in cosmetics and their concentration is not limited at the moment. However, the European Scientific Committee on Consumer Safety issued its positive opinion on the safety of retinol, retinyl acetate and retinyl palmitate, but specifying which are the concentrations that can be considered safe (less than 0.05% in body lotions and 0.3% in hand and face creams as well as rinse-off products) [9].

Therefore, analytical methods for the simultaneous determination of retinoids in cosmetics samples are required in order to perform the quality control for both to achieve the desired efficacy and to ensure the consumers' safety.

Retinoids have been determined in different matrices, such as food, pharmaceutical preparations and biological fluids, employing liquid chromatography (LC) coupled to tandem mass spectrometry, and with ultraviolet (UV) detection, fluorescence spectrometry, capillary electrophoresis and electrochemical detection [10–12]. Regarding cosmetic products, there are no official methods for the simultaneous determination of retinol and its derivatives in cosmetic samples, and the published analytical methods related to the quantification of some retinoids are scarce. Retinol has been determined in different cosmetic matrices by LC-UV [13] and UV spectrophotometry [5], whereas some LC-UV methods have been described to determine retinyl palmitate, as the most commonly used retinol-derivative [14–16]. On the other hand, about the simultaneous determination of different retinoids, only combinations of a few retinoids have been described: retinol, retinal and retinoic acid by differential pulse voltammetry and LC-UV [17]; retinol, retinyl palmitate and retinoic acid by LC-UV [18]; retinol, retinyl palmitate and retinyl acetate by LC-UV [19]; and retinyl acetate and retinyl palmitate by cyclic voltammetry [20]. It should be also noted that retinyl propionate has not been determined in none of these publications, and moreover these methods require the use of organic solvents such as methanol, n-hexane, dichloromethane, or chloroform in both sample preparation and analysis.

Considering all the above, the main aim of this work is to develop a rapid and simple analytical method for the simultaneous quantification of the five most common retinoids (Table 1) in cosmetic samples. The proposed method is based on LC-UV technique with a rapid treatment of the sample, by simple dilution with ethanol, a more benign solvent than those usually employed [21], and without requiring multistage sample preparation procedures. It provides similar or improved analytical features than the previous works with similar purpose, allowing to perform both pre- and in-market analytical control in the cosmetic sector.

Table 1. Chemical structure and some relevant data of the target analytes.

Retinoid	Chemical Structure	N° CAS	LogP _{ow} ^a
Retinol (ROL)		68-26-8	6.078
Retinal (RAL)		116-31-4	6.376
Retinyl Acetate (RAc)		127-47-9	7.190
Retinyl Propionate (RPr)		7069-42-3	7.699
Retinyl Palmitate (RPal)		79-81-2	14.322

^a Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 2022–2021 ACD/Labs).

2. Materials and Methods

2.1. Reagents and Samples

Retinol (ROL) ≥ 95%, all-trans-retinal (RAL) ≥ 98%, retinyl propionate (RPr) ≥ 98%, retinyl acetate (RAc) pharmaceutical secondary standard from Sigma-Aldrich (St. Louis, MO, USA); and retinyl palmitate (RPal) 1.7 M.I.U./g from Alfa Aesar (Kandel, Germany) were used as standards. The chemical structures and relevant data are presented in Table 1.

LC-grade absolute ethanol (EtOH) from Panreac (Barcelona, Spain), and deionized water obtained from a Connect water purification system provided by Adrona (Riga, Latvia) were used to prepare the chromatographic mobile phase. Considering the chemical features of the analytes, butylhydroxytoluene (BHT) from Guinama S.L. (Valencia, Spain) was used as antioxidant reagent in the standard and sample solutions.

Seven commercial cosmetic samples of different formulation (i.e., a night moisturizer (S1), a rejuvenating face mask (S2), a hand cream (S3), an anti-age serum (S4), and three anti-age creams (S5–S7)) were purchased at local stores and analyzed by the proposed method. Due to confidentiality reasons, their brands are not mentioned. A summary of the declared retinoid in the cosmetic label is presented in Table 2.

Table 2. Summary of the declared retinoids in the cosmetic label.

Sample Type	Sample Code	Retinoid				
		ROL	RAL	RAc	RPr	RPal
Night moisturizer	S1	X			X	
Rejuvenating face mask	S2	X			X	
Hand cream	S3			X		X
Anti-age serum	S4					X
Anti-age cream 1	S5	X	X			X
Anti-age cream 2	S6	X				
Anti-age cream 3	S7					X

2.2. Apparatus

An Agilent 1220 Infinity LC system with a degasser, a binary pump, an autosampler with up to 100 μ L injection volume, a thermostated column oven, and a variable wavelength UV/V is detector was used. Data acquisition and processing were performed with ‘Agilent LC ChemStation v.04.03’ software from Agilent Technologies (Palo Alto, CA, USA).

A ZX3 vortex mixer from VELP Scientifica (Usmate Velate, Italy) and an Ultrasons-HD ultrasonic bath from JP Selecta (Barcelona, Spain) were used to ease the dispersion of the samples.

2.3. Proposed Method

2.3.1. Preparation of Standards and Sample Solutions

A solution of EtOH (containing 50 μ g mL^{−1} of BHT as antioxidant) was used as solvent (hereinafter, ethanolic solution). A stock standard solution containing 100 μ g mL^{−1} of the five target retinoids was prepared. This stock solution was stable for at least one month at −20 °C. From this solution, working calibration solutions (1–10 μ g mL^{−1}) were freshly prepared by proper dilution with the ethanolic solution.

Sample solutions were prepared by weighing 0.01–0.5 g of cosmetic sample into a 10-mL volumetric flask. For this purpose, a small amount of the sample was placed on the tip of a Pasteur pipette and carefully deposited on the inner walls of the volumetric flask. Then, it was dissolved and diluted up to the line with the ethanolic solution. The samples that were not completely dissolved were stirred by vortex and/or sonication (ca. 5 min) to carry out the leaching of the analytes and the solutions were filtered throughout 0.22- μ m nylon filters to remove non-soluble compounds. Finally, both standard and sample solutions were placed into amber injection vials for LC-UV analysis.

2.3.2. Chromatographic Analysis

A LiChrospher® 60 RP-select B (250 mm length, 4 mm I.D., 5 μ m particle size) column from Merck (Darmstadt, Germany) was used for the chromatographic separation. Twenty microliters of standard or sample solution were injected into the chromatographic system. Mobile phase consisted of water (solvent A) and ethanol (solvent B), and the pump supplied the following gradient program: 0 to 8 min, 55% solvent B; 8 to 16 min linear gradient to 83% solvent B; 16 to 16.1 min linear gradient to 100% solvent B, held for 8 min; and 24 to

24.1 min return to 55% solvent B and held for 6 min. The run time was 30 min. The flow rate was set at 1 mL min⁻¹ and the column temperature was maintained constant at 40 °C. Given that the absorption maximum of the analytes lies at 325 nm except for RAL, whose absorption maximum lies at 380 nm, a compromise wavelength of 350 nm was selected. Figure 1 shows a chromatogram obtained according to the proposed method.

Figure 1. Chromatogram of a standard solution containing the analytes at 10 µg mL⁻¹ obtained according to the proposed method.

3. Results and Discussion

3.1. Study of the Chromatographic Variables

With the aim of obtaining the best chromatographic resolution in the shortest analysis time, different variables, such as chromatographic column, oven temperature and composition of mobile phase were studied. To carry out these studies, a standard solution containing ROL and RAL at 25 µg mL⁻¹ was firstly used due to their structural similarity. Once ROL and RAL were chromatographically resolved, a standard solution containing all the target analytes at 25 µg mL⁻¹ was employed to separate the most non-polar analytes (i.e., RAc, RPr and RPal). Water and EtOH were selected as solvents for the mobile phase. Non-buffered water was used since the target analytes are not ionizable and, therefore, not affected by pH. EtOH was preferred as organic modifier over other typical solvents, such as methanol or acetonitrile, due to its less toxic nature, being the proposed method more environmentally friendly than those previously reported.

Different reversed-phase columns were used to study the effect of the column packing, while maintaining constant the composition of the mobile phase (i.e., 60% of EtOH by isocratic elution) and the oven temperature (i.e., 25 °C). First, a Purospher® STAR RP-18 endcapped (125 mm length, 4 mm I.D., 5 µm particle size) was tested, but ROL and RAL were completely overlapped. In order to separate them, less elution force was tested (i.e., 50% of EtOH), but the analytes did not elute in reasonable chromatographic times. Consequently, a LiChrospher® 100 RP-18 (250 mm length, 4 mm I.D., 5 µm particle size) was then used in order to observe the effect of the column length. The increase of the column length did not achieve to resolve ROL and RAL peaks in a reasonable time. For this reason, alternative column packings (i.e., C8 columns) were tested. In this sense, when a LiChrospher® 60 Select-B (250 mm length, 4 mm I.D., 5 µm particle size) was used, ROL and RAL peaks were resolved in approximately 20 min. In order to reduce these retention times, a LiChrospher® 60 Select-B (125 mm length, 4 mm I.D., 5 µm particle size) was tested

but ROL and RAL peaks overlapped again (see Figure 2). Thus, a LiChrospher® 60 Select-B (250 mm length, 4 mm I.D., 5 µm particle size) was selected for further analysis.

Figure 2. Chromatograms of a standard solution containing ROL and RAL at $10 \mu\text{g mL}^{-1}$ obtained using a (a) LiChrospher® 60 Select-B (125 mm $\times$ 4 mm, 5 µm), and (b) a LiChrospher® 60 Select-B (250 mm $\times$ 4 mm, 5 µm).

Regarding the oven temperature, different values between 25 and 45 °C were tested in order to improve the resolution of the peaks and to reduce the retention times. The composition of the mobile phase was set at 60% of EtOH by isocratic elution, in a LiChrospher® 60 Select-B (250 mm length, 4 mm I.D., 5 µm particle size). As the oven temperature increased, the resolution of the peaks, provided by the software, decreased from 2.43 to 1.54, and the retention time got lower (see Figure 3). Nevertheless, when the oven temperature was set at 45 °C, a partial overlapping between ROL and RAL was observed due to the asymmetry of both peaks even though resolution was greater than 1.5. Thus, 40 °C was selected for further analysis.

Finally, once column packing and oven temperature were selected, a gradient elution program was optimized in order to achieve the best resolution for all the target analytes. In this sense, starting with 55% of EtOH for 8 min, and then a linear gradient to 83% for other 8 min, improved the resolution of ROL and RAL peaks making them less wide. Moreover, this linear gradient allowed to elute RAc and RPr with a good resolution. RPal was finally eluted when the gradient turned to 100% of EtOH due to its high lipophilic character.

Figure 3. Chromatograms of a standard solution containing ROL and RAL at 10 $\mu\text{g mL}^{-1}$ obtained at (a) 25 °C, (b) 30 °C, (c) 35 °C, (d) 40 °C and (e) 45 °C.

3.2. Sample Treatment

Samples with different cosmetic formulation were assayed (i.e., a night moisturizer, a rejuvenating face mask, a hand cream, an anti-age serum, and three anti-age creams)

in order to find a green sample treatment that was common for all of them, regardless of their matrix.

EtOH containing $50 \mu\text{g mL}^{-1}$ of BHT was used as a solvent due to several reasons: (i) the target retinoids are highly soluble at working concentration levels in this solvent, (ii) they are stabilized by the antioxidant action of BHT [22], (iii) they are satisfactorily leached without the necessity of total dissolution, and (iv) it is a suitable solvent for LC-UV.

This procedure only required a brief shaking assisted by ca. 5 min of vortex and/or sonication in order to achieve the complete leaching of the target analytes to the organic phase from the cosmetic matrix. It should be mentioned that the retinoids remained stable during the sonication of the sample solution for at least 10 min.

3.3. Analytical Figures of Merit of the Developed Method

Analytical parameters of the proposed method were evaluated in terms of linearity, limits of detection and quantification, and repeatability. These results are summarized in Table 3.

Table 3. Analytical figures of merit of the developed method.

Retinoid	ILOD ^a ($\mu\text{g mL}^{-1}$)	ILOQ ^a ($\mu\text{g mL}^{-1}$)	MLOD ^b (%, w/w)/ 10^{-4}	MLOQ ^b (%, w/w)/ 10^{-4}	Precision (RSD, %) ^c					
					Intra-Day			Inter-Day		
					1 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	25 $\mu\text{g mL}^{-1}$	1 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	25 $\mu\text{g mL}^{-1}$
ROL	0.05	0.2	1.1	3.6	8.8	2.5	0.8	7.4	2.9	0.9
RAL	0.3	1.0	5.9	19.3	10.0	1.5	0.7	8.1	5.9	5.2
RAc	0.03	0.08	0.5	1.5	8.9	1.9	0.8	5.9	4.6	1.5
RPr	0.02	0.06	0.3	1.1	13.8	1.8	0.8	6.2	4.6	1.4
RPal	0.02	0.08	0.5	1.5	11.4	2.7	1.1	9.8	4.6	2.4

^a ILOD: instrumental limit of detection; ILOQ: instrumental limit of quantification; calculated as 3 and 10 times, respectively, the signal-to-noise ratio. ^b MLOD: method limit of detection; MLOQ: method limit of quantification (i.e., in the cosmetic sample). ^c RSD: relative standard deviation ($n = 5$).

The linearity reached at least up to $100 \mu\text{g mL}^{-1}$ for all the analytes, obtaining good coefficients of determination ($R^2 > 0.998$). The working range to prepare the external calibration was established from 1 to $10 \mu\text{g mL}^{-1}$.

The instrumental limits of detection (ILOD) and quantification (ILOQ) were calculated as 3 and 10 times the signal-to-noise ratio (S/N), respectively, of a standard solution at $1 \mu\text{g mL}^{-1}$. They were at the low $\mu\text{g mL}^{-1}$ level, ranging from 0.02 to $0.3 \mu\text{g mL}^{-1}$ and from 0.06 to $1.0 \mu\text{g mL}^{-1}$, respectively. The method limits of detection (MLOD) and quantification (MLOQ), considering the maximum recommended weight of sample and its dilution, ranged from 0.3×10^{-4} to 5.9×10^{-4} % w/w and 1.1×10^{-4} to 19.3×10^{-4} % w/w, respectively. The low limits and wide linear range allow to determine the target analytes in a wide range of concentrations.

The precision of the method was evaluated by applying the developed method to five independent replicates of standard solution containing all the target analytes at three concentration levels (i.e., 1, 10 and $25 \mu\text{g mL}^{-1}$) on the same day (intra-day precision) and on five consecutive days (inter-day precision). As it is shown in Table 3, the results expressed as relative standard deviation (RSD, %) ranged from 0.7 to 13.8%, showing the good precision of the proposed method.

System suitability test was carried out to ensure the chromatographic parameters are acceptable for the intended application. In this sense, Table 4 shows values of retention time, asymmetry factor, number of theoretical plates per meter and retention factor for each analyte in a $10 \mu\text{g mL}^{-1}$ standard solution.

Table 4. System suitability test for a standard solution of $10 \mu\text{g mL}^{-1}$ applying the proposed method.

Analytes	t_R (min) ^a	A_s ^b	N/m ^c	k ^d
ROL	14.35	0.72	72,080	5.08
RAL	14.97	0.83	88,700	5.34
RAc	17.09	0.77	229,896	6.24
RPr	17.66	0.79	298,932	6.48
RPal	19.85	0.95	1,371,136	7.41

^a t_R : retention time. ^b A_s : Asymmetry factor calculated according to IUPAC recommendations. ^c N/m: Number of theoretical plates per meter. ^d k: retention factor.

3.4. Analysis of Commercially-Available Cosmetic Samples

Seven commercially available cosmetic samples (samples S1–S7) were prepared by triplicate as specified in Section 2.3.1 and injected into the LC system under the selected conditions. The results obtained by using the proposed method are shown in Table 5.

Table 5. Retinoids content in seven commercial cosmetics applying the proposed method.

Sample	Found Amount (% w/w) (n = 3)				
	ROL	RAL	RAc	RPr	RPal
S1	<MLOD	<MLOD	<MLOD	0.2735 ± 0.0011	<MLOD
S2	2.57 ± 0.07	<MLOD	<MLOD	0.432 ± 0.012	<MLOD
S3	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
S4	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
S5	0.00363 ± 0.00005	0.00290 ± 0.00005	<MLOD	<MLOD	0.00121 ± 0.00007
S6	0.267 ± 0.006	<MLOD	<MLOD	<MLOD	<MLOD
S7	<MLOD	<MLOD	<MLOD	<MLOD	0.301 ± 0.004

Subsequently, the accuracy and possible matrix effects were evaluated by performing recovery studies. Two fortifications were done to get $2 \mu\text{g mL}^{-1}$ and $4 \mu\text{g mL}^{-1}$, respectively, of the standards in the final sample solution. To achieve this, samples were weighed as indicated in Section 2.3.1, the corresponding volume of the $100 \mu\text{g mL}^{-1}$ standard solution was added to fortify the sample and the appropriate dilution was made. The process was carried out in triplicate for each level of fortification and the fortified samples were analyzed according to the proposed method. The obtained recovery values, shown in Table 6, ranged from 79 and 117%, revealing negligible matrix effects and that external calibration can be used as described in the proposed method. Figure 4 shows, as example, the chromatograms obtained for the cosmetic sample S5 (a) unfortified and (b) fortified at $4 \mu\text{g mL}^{-1}$.

Table 6. Recovery values of the target compounds in the analyzed cosmetic samples.

Sample	Recovery Values (%) (n = 3)									
	ROL		RAL		RAc		RPr		RPal	
	$2 \mu\text{g mL}^{-1}$	$4 \mu\text{g mL}^{-1}$	$2 \mu\text{g mL}^{-1}$	$4 \mu\text{g mL}^{-1}$	$2 \mu\text{g mL}^{-1}$	$4 \mu\text{g mL}^{-1}$	$2 \mu\text{g mL}^{-1}$	$4 \mu\text{g mL}^{-1}$	$2 \mu\text{g mL}^{-1}$	$4 \mu\text{g mL}^{-1}$
S1	103 ± 2	102 ± 2	94 ± 9	97 ± 5	98 ± 1	97 ± 2	96 ± 5	96 ± 1	95 ± 2	99 ± 6
S2	102 ± 2	99 ± 3	103 ± 3	104 ± 3	92 ± 2	93 ± 2	94 ± 2	94 ± 2	102 ± 2	95 ± 2
S3	93 ± 3	99 ± 1	87 ± 9	114 ± 2	86 ± 1	90 ± 1	89 ± 1	91 ± 1	89 ± 1	91 ± 2
S4	108 ± 5	101 ± 2	95 ± 1	87 ± 1	93 ± 4	90 ± 1	97 ± 5	95 ± 1	93 ± 5	91 ± 1
S5	93 ± 2	93 ± 4	95 ± 3	85 ± 8	79 ± 1	83 ± 4	82 ± 1	86 ± 4	90 ± 2	90 ± 3
S6	105 ± 2	101 ± 2	117 ± 2	107 ± 3	93 ± 2	93 ± 1	92 ± 2	92 ± 1	97 ± 4	92 ± 1
S7	102 ± 4	101 ± 3	107 ± 2	103 ± 5	92 ± 4	93 ± 2	91 ± 3	92 ± 2	91 ± 6	91 ± 2

Figure 4. Chromatogram of sample S5 obtained according to the proposed method: (a) unfortified and (b) fortified at $4 \mu\text{g mL}^{-1}$.

According to the labels, the seven commercial cosmetic products analyzed should contain several retinoids (see Table 2), but Table 5 shows that some of the retinoids indicated in the label were undetected. As described above, matrix effects were negligible in cosmetic samples and low limits of detection were achieved (see Table 3). Given that samples and standards were kept in right conditions to avoid degradation and that both were injected in the same chromatographic conditions, results indicate that the analytes that have not been detected must be, if present, in a very low concentration, being their efficacy as active ingredients questionable.

3.5. Advantages of the Proposed Method: Evaluation of the Greenness of the Proposed Sample Preparation

A comparison of the proposed method with other previously published methods with similar purpose is presented in Table 7. The proposed method allows to determine simultaneously the highest number of retinoids (i.e., retinol and four derivatives), whereas in the other methods only a maximum of three retinoids is determined. RPr was not determined in any of the previously published methods.

Table 7. Comparison of the proposed approach with other methods for the determination of retinoids in cosmetic products.

Analytes	Matrix	Extraction Solvent ^a	Instrumental Technique ^b	Ref.
RPal	Creams and lotions	Extraction with supercritical CO ₂ and collection in THF:MeOH 4:1	LC-UV	[14]
ROL, RAL	Anti-acne and anti-wrinkle creams and gel	IPA:MeOH 1:1	DPV and LC-UV	[17]
ROL, RAc, RPal	Body lotions and skin nourishing creams	MeOH:Hex 1:1	LC-UV	[15]
RPal	Gel-cream preparations	IPA	LC-UV	[16]
ROL, RAL, RPal	Creams and lotions	Hex:IPA:AcOEt 1:1:1	LC-UV	[18]
ROL	Serums	CHCl ₃	UV	[5]
ROL, RAc, RPal	Serums, creams, filler fluid, lip gloss and fixing spray	ACN, Hex	LC-UV	[19]
RAc, RPal	Face hands creams, refreshing cleaning body milk	0.1 M LiClO ₄ in acetone	CV	[20]
ROL, RAL, RAc, RPr, RPal	Moisturizer, face mask, hand creams, anti-age serum	EtOH	LC-UV	This work

^a ACN: acetonitrile; AcOEt: ethyl acetate; EtOH: ethanol; Hex: n-hexane; IPA: isopropyl alcohol; MeOH: methanol; THF: tetrahydrofuran. ^b CV: cyclic voltammetry; DPV: differential pulse voltammetry; LC: liquid chromatography; UV: ultraviolet spectroscopy.

Moreover, these other methods require the use of organic solvents such as tetrahydrofuran, n-hexane, or ethyl acetate. As a consequence, these approaches result less environmentally friendly than the proposed method.

A wide variety of methodologies to evaluate the greenness of analytical methods have been described, such as the Analytical Eco-Scale [23], the Green Analytical Procedure Index (GAPI) [24] and the Green Certificate [25]. The proposed sample preparation has been evaluated according to the Green Certificate, which assigns penalty points to those aspects of the method that differ from the principles of Green Analytical Chemistry [26] (i.e., use of reagents, consumed energy, operational hazards and generated wastes), establishing a scale from A to G. Penalty points assigned to the proposed method are: (i) 2.5 points due to the use of 10 mL per sample of ethanol; (ii) 3.8 points due to the generated waste of 10 mL per sample of ethanol; (iii) 2 points due to the energy consumption of the ultrasound-assisted leaching, and (iv) 0 points due to the low energy consumption of the vortex instrument (i.e., <0.1 kWh). Hence, with a total of 8.3 penalty points, the sample treatment is considered a class A method (less than 10 penalty points), being in accordance with the principles of Green Analytical Chemistry.

4. Conclusions

In this work, a simple and rapid method for the simultaneous determination of vitamin A and four of its derivatives in hygiene and personal care products is presented. This method based on LC-UV after a simple vortex and/or ultrasound-assisted leaching of the target compounds has shown high levels of linearity, at least up to 100 µg mL⁻¹; high precision with RSD values below 14 % and high sensitivity with low MLODs ranging

between 0.3×10^{-4} and 5.9×10^{-4} % w/w, which are enough to monitor these compounds in cosmetic products. Recovery values around 100% showed that matrix effects were negligible and, thus external standard calibration is appropriate for quantification.

The method was successfully applied to commercial cosmetic samples, it is effective for the quantification of these analytes and therefore it can be useful for manufacturers to ensure the quality, efficacy and safety of cosmetic products.

Additionally, the proposed sample treatment is safe for both the operator and the environment, being in line with current trends set by Green Analytical Chemistry.

Author Contributions: Conceptualization, V.V.-G., C.A., J.L.B., A.C. and A.S.; methodology, V.V.-G., A.C. and A.S.; validation, V.V.-G., C.A., J.L.B., A.C. and A.S.; formal analysis, S.C.-O.; investigation, V.V.-G., S.C.-O. and C.A.; resources, J.L.B., A.C. and A.S.; data curation, S.C.-O.; writing—original draft preparation, V.V.-G. and C.A.; writing—review and editing, J.L.B., A.C. and A.S.; supervision, J.L.B., A.C. and A.S.; project administration, A.C. and A.S.; funding acquisition, A.C. and A.S. All authors have read and agreed to the published version of the manuscript.

Funding: Authors thank the Generalitat Valenciana and the European Social Fund for the predoctoral grant of V.V.-G. (ACIF/2020/107); and the Spanish Ministry of Universities for the predoctoral grant of C.A. (FPU19/04239).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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