Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue



Cite this: *Anal. Methods*, 2023, **15**, 951

# Kinetic spectrophotometric assay for the determination of vitamin C in cosmetics following ultrasound-assisted emulsification†

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In this work, a new analytical approach based on ultrasound-assisted emulsification followed by a photoreaction with methylene blue (MB) and kinetic analysis by UV-vis spectrophotometry has been developed for the determination of L-ascorbic acid (AA) in cosmetic samples. The emulsification of cosmetic samples results in a transparent solution that allows an easy and rapid quantitation by UV-vis spectrophotometry. The emulsified sample is mixed with a MB aqueous solution and this mixture is subjected to irradiation with a tungsten lamp for 5 min (fixed-time kinetic assay). A reduction in the MB absorbance intensity at 664 nm occurs as the concentration of AA increases. The observed change in absorbance intensity was used for calibration and further quantitation using the relationship of absorbance logarithm *vs.* AA concentration (μg $mL^{-1}$). In order to achieve an optimal response, different parameters involved in the reaction between AA and MB were fully investigated. Under optimal conditions, the limits of detection and quantification were 0.04 μg $mL^{-1}$ and 0.15 μg $mL^{-1}$, respectively. Repeatability and reproducibility, expressed as relative standard deviation, were in the range of 0.4–0.6% and 0.6–1.5%, respectively. Finally, the proposed method was applied to the analysis of 15 cosmetic samples, namely, (i) 12 samples without AA, which were used to carry out recovery studies, obtaining results in the range of 97.5–100.7%; (ii) 3 serum samples containing pure AA among their ingredients, which were used for AA stability studies.

Received 2nd November 2022
Accepted 20th January 2023

DOI: 10.1039/d2ay01795d

rsc.li/methods



## Introduction

Vitamin C or L-ascorbic acid (AA) is a classic ingredient in cosmetics due to its antioxidant activity and acidity, which is currently in vogue. Thus, AA has been used as a preservative enhancer and pH adjuster, and more recently as an anti-aging and photo-protecting agent. It is considered that AA, applied topically, neutralizes reactive oxygen species, inhibits tyrosinase activity reducing hyperpigmentation, melasma and sunspots, and facilitates cellular differentiation of keratinocytes and dermal-epidermal cohesion.[1]

The concentrations of AA typically used in cosmetics in the early 2000s, as well as the number of formulations containing this ingredient, according to the U.S. Food and Drug Administration (U.S. FDA), can be found in the "final report of the safety assessment of L-ascorbic acid, calcium ascorbate, magnesium ascorbate, magnesium ascorbyl phosphate, sodium ascorbate, and sodium ascorbyl phosphate as used in cosmetics".[2] Considering the product category, AA was mainly used in hair dyes and hair colors at concentrations between 0.3% and 0.6%. Besides, it should be noted that in the early 2000s, those skin care preparations containing AA in their formulations were a minority group (*i.e.*, only 0.2–1% of products) with concentrations between a few μg $g^{-1}$ and 10%. However, at present, there is increasing interest in skin care cosmetics, mainly creams and serums with a high concentration of AA or its derivatives. In these cosmetic formulations, AA is used as an anti-aging and brightening agent. The concentration of AA in these skin care cosmetics depends on its formulation. The maximum recommended concentration of AA in cosmetic formulations is 20%.[3] A higher concentration of AA is not recommended since it can cause skin irritation.[4]

The main problem with the use of AA as an active ingredient in cosmetics is its instability, since it is easily degraded or oxidized in the presence of light, oxygen, metals, high temperatures or pH. In fact, many cosmetics that contain AA as a strategic ingredient actually include AA derivatives such as 3-*O*-ethyl L-ascorbic acid, L-ascorbyl palmitate, sodium L-ascorbyl phosphate or L-ascorbyl 2-*O*-α-glycoside, which are more stable but display lower antioxidant capacity.[5] Nevertheless, AA stands out as an active ingredient in cosmetic formulations, enhancing its stability through improved encapsulation and delivery systems.[1]

The determination of AA in a wide range of concentrations (from a few μg $g^{-1}$ up to 200 mg $g^{-1}$) is fundamental in

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d2ay01795d

cosmetics formulations, not only for their quality control, but also for the proposal of new improved formulae. Analytical methods for determining AA in cosmetics usually involve liquid chromatography with different detectors such as UV-vis,[6–9] chemiluminescence,[10] MS/MS[11] and electrophoresis.[12] In all cases, it is necessary to extract the analyte including different stages of filtration and/or centrifugation. In general, these procedures are time-consuming and difficult to adapt to routine laboratories. In contrast, spectrophotometric methods are particularly attractive for the cosmetic industry due to their speed and simplicity.[13] Although they have not been yet applied for the analysis of AA in cosmetics, different colored reagents such as 2,6-dichlorophenolindophenol (DCIP), dimethoxyquinoline (DMDQ), ninhydrin, methylene blue, (MB) *etc.*, which can react with AA resulting in absorbance changes, can be used for the design of spectrophotometric methods.[14–16]

Nevertheless, cosmetics are very complex matrices, and the successful application of spectrophotometric methods requires the development of innovative procedures for sample preparation. In this regard, ultrasound-assisted emulsification allows the formation of stable and completely transparent emulsions with cosmetic samples, providing a simple and rapid sample preparation strategy that allows the use of spectrophotometry techniques for quantitation.[17]

Despite the simplicity that spectrophotometric methods offer for routine analysis, they lack enough selectivity. Then, in many cases it is necessary to make prior separations and/or use masking agents to eliminate interference, which reduces their advantages. An alternative to these classic strategies for eliminating interference is the design of kinetic methods using a coloured reagent as an indicator. The main analytical characteristic of kinetic methods is their high selectivity. Interference separation/removal processes prior to analysis are not usually necessary. Thus, kinetic methods are useful to solve mixtures of related compounds.[18]

In this work, a spectrophotometric kinetic method was developed for the rapid and simple determination of pure AA in cosmetics. The proposed approach was adapted to a wide range of AA concentrations that can be found in this type of sample. For this purpose, ultrasound-assisted emulsification combined with a photoreaction between AA and MB was carefully studied. The application of the new method to the determination of pure AA in cosmetic samples was demonstrated.

# Experimental

### Instrumentation

An UV-vis molecular absorption spectrophotometer Cary 300 series Agilent Technologies (Santa Clara, California, USA) equipped with quartz cuvettes was used for absorbance measurements. A Sartorius microbalance, model MC 5 (Göttingen, Germany), a Scaltec precision balance, model SBC 32 (Heiligenstadt, Germany), a Hielscher cup-horn sonoreactor, model UTR200 (Teltow, Germany) (200 W, 24 kHz) and a vortex V-1 plus (BioSan, Riga, Latvia) were used for the preparation of cosmetic samples.

### Reagents and samples

A stock standard (500 mg $L^{-1}$) and working standard solutions of AA were daily prepared by dissolving the corresponding amount of AA (Merck, Darmstadt, Germany) in ultrapure water. Other reagents used were: methylene blue, MB (Analema Vorquímica, Vigo, Spain), sodium dodecylsulfate, SDS (Sigma Aldrich, Steinheim, Germany), Tween 80 (Sigma), Triton X-100 (Merck), polyvinylpyrrolidone, PVP (Fluka, Steinheim, Germany), polyethylene glycol, PEG (Sigma Aldrich), acetic acid (Analema Vorquímica, Vigo, Spain), formic acid (Merck), hydrochloric acid (VWR Prolabo, Fontenay-sous-Bois, France), nitric acid (VWR Prolabo), L-ascorbyl palmitate (Sigma Aldrich), sodium L-ascorbyl phosphate (Sigma Aldrich), L-ascorbyl 2-*O*-α-glycoside (Sigma Aldrich), α-tocopherol (Sigma Aldrich), citric acid (Sigma Aldrich), salicylic acid (Panreac, Barcelona, Spain), ethanol (VWR) and sodium hydroxide (VWR).

All reagents used were of analytical grade or higher. The ultrapure water was obtained from a Merck Millipore purification system, model Simplicity, and resistivity 18.2 mΩ cm (Darmstadt, Germany).

15 samples of commercial skin care cosmetics (creams, serums, and gels), with and without AA, were analyzed. Their declared composition can be found in the ESI (Table S1†).

### Ultrasound-assisted emulsification procedure

Between 10 and 15 mg of cosmetic sample were accurately weighed in an Eppendorf® tube. Then, 1.5 mL of surfactant solution (1% m/v SDS prepared in 2% v/v acetic acid) were added. The mixture was vortexed for 10–15 s to disperse the sample. Subsequently, the Eppendorf® tube is placed in an ultrasonic reactor. 6 samples were simultaneously sonicated for 3 minutes at an amplitude of 50%. After emulsification, completely transparent solutions were obtained (Fig. S1†). Standard and blank solutions were treated in the same way.

### Measurement procedure

An aliquot of the emulsion (between 10 μL and 1.5 mL, depending on the concentration of AA in the analysed sample) was transferred to a 3.5 mL capacity cuvette and the required volume of ultrapure water was added to complete 1.5 mL. Finally, 1.5 mL of 0.04 mM MB solution containing SDS and acetic acid was added. In all cases, the final concentrations of AA, SDS and acetic acid in the cuvette were 2 μg $mL^{-1}$, 0.5% m/v and 1% v/v, respectively. The content of the cuvette was rapidly homogenized and irradiated under a tungsten lamp in a closed system for 5 min (Fig. S2†). After that, the absorbance was measured at 664 nm (fixed time kinetic method). Standards and blanks were treated in the same way. For calibration, the logarithm of the absorbance (Log(Abs)) measured after 5 min of irradiation *vs.* AA concentration (μg $mL^{-1}$) was used.

# Results and discussion

### Effect of ultrasound irradiation on AA stability

Cavitation is the phenomenon that gives rise to the main effects of ultrasound, including the formation of radicals and other

 

reactive species that could oxidize the AA.[19] Therefore, the influence of ultrasound on the stability of an aqueous AA solution in acidic media and in the presence of different surfactants or stabilizers such as PVP, SDS, PEG, Triton X-100 and Tween 80 was initially tested. For this purpose, mixtures of acetic acid and surfactant/stabilizer containing aqueous solution of AA (2 μg $mL^{-1}$), were subjected to sonication or mechanical stirring at different times (between 0.5 and 5 min). Then, each solution was transferred to a UV quartz cuvette following the aforementioned measurement procedure. In none of the cases AA was degraded, since it preserved its capacity to react with MB within the time range tested (Fig. S3†). No significant differences were observed between sonication and mechanical stirring. However, it was observed that the surfactant or stabilizer has a strong influence on the reaction rate, so this variable was exhaustively studied during the optimization experiments.

### Preliminary studies on matrix effects

The emulsion formed containing the cosmetic sample, surfactant or stabilizer and a diluted acid could influence the spectrum of MB and/or the rate of the reaction between AA and MB. To study this effect, a cream without AA (*i.e.* Babaria® moisturizing oil free facial cream) was used as a model of the cosmetic matrix, SDS was used as the surfactant and acetic acid for the acidic medium. First, the UV-vis spectra of a MB aqueous solution with and without emulsion were obtained (Fig. 1). No significant differences were observed between both spectra, showing that the presence of emulsion in the medium neither caused a shift of the peak nor changes in absorbance.

In addition, to assess the influence of the emulsion on the reaction rate between AA and MB, spectra were obtained by adding AA to the sample at a concentration of 2 μg $g^{-1}$ and the mixture was irradiated for 5 min under a tungsten lamp in a closed system before absorbance measurement. No differences were observed between the spectra with and without emulsion (Fig. 1), so it can be concluded that there is no matrix effect on the reaction rate under the experimental conditions used.

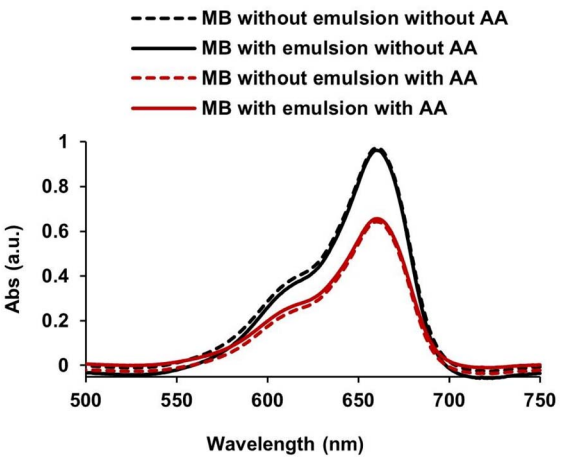


Fig. 1 MB UV-vis spectra with and without emulsion in the presence (sample) and absence (blank) of AA (0.04 mM MB; 0.5% m/v SDS; 1% v/v acetic acid; 2 μg $g^{-1}$ AA).

### Study of reaction conditions

For the development of a kinetic method, it is necessary to work in the kinetic region. If the reaction rate is too high, the equilibrium region is reached quickly, hence preventing the reaction medium from suitable mixing before reaching this region. On the other hand, if the reaction is too slow, the sample throughput would be worsened. Hence, it is necessary to establish suitable reaction conditions that enable a mixing step, appropriate sample throughput as well as good precision (usually, precision is worst for kinetic methods in comparison to equilibrium methods).

In this case, the reaction between AA and MB is influenced by different variables such as the presence of light, an acidic medium that can act as a catalyst for the reaction and the presence of surfactants or stabilizers.[20] The kinetics of this reaction is complex since it depends to a large extent on the medium composition.[21] In order to facilitate the determination of AA, pseudo-order conditions concerning the rest of the components, *i.e.* MB, acid, and surfactant or stabilizer, were used.

**Type of light.** Light activates the photochemical reaction between AA and MB, and its intensity has an important effect on the reaction rate.[16] To evaluate the influence of this parameter,

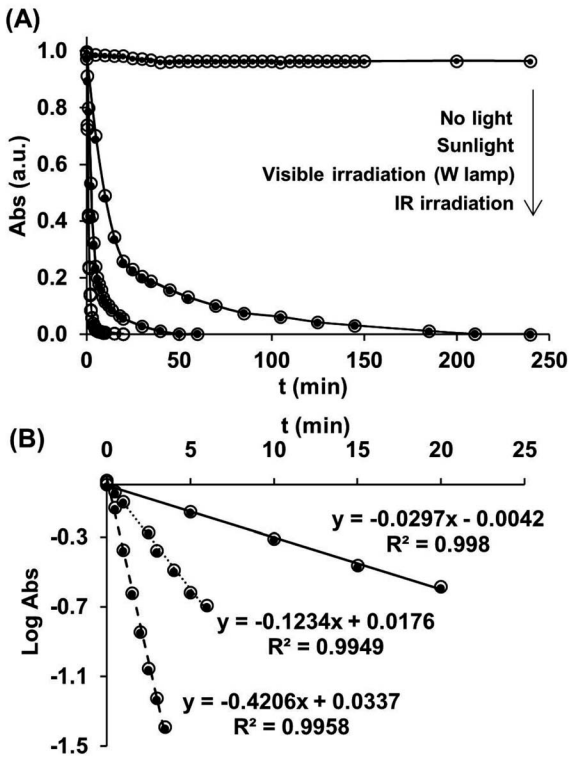


Fig. 2 (A) Kinetic curves with (•) and without (o) emulsion in the absence of light, under sunlight, and under irradiation of a tungsten lamp (W lamp) and IR lamp; (B) Log(Abs) *vs.* *t* (min) under sunlight (—), W lamp (· · · ·), and IR lamp (— —). (0.04 mM MB; 0.5% m/v SDS; 1% v/v acetic acid; 2 μg $mL^{-1}$ AA).

kinetic curves were obtained for a 2 μg $mL^{-1}$ AA solution using different types of light: absence of light, sunlight, 12 V/50 W tungsten lamp (visible radiation), and 230 V/150 W IR lamp. Fig. 2(A) shows the results obtained. As can be seen, the absence of light implies that after 240 min, the absorbance only decreases by 3.1% with respect to the initial absorbance. Under sunlight, equilibrium *i.e.*, the total disappearance of MB absorbance resulting in colourless LMB, is reached in about 210 min.

As shown, the reaction rate using the tungsten lamp and IR lamp is faster, and equilibrium conditions were reached in 50 min and 15 min, respectively. Furthermore, the precision expressed as relative standard deviation (RSD, %) at 5 min was 6.6%, 16% and 0.3% for sunlight, the IR lamp and the tungsten lamp, respectively. Based on these results, the tungsten lamp was selected as it provides a reaction rate that enables a suitable sample throughput for designing a kinetic method, being also the one with the best RSD value. As shown in Fig. 2(B), first-order kinetics with respect to AA under the conditions used (except in the absence of light) is observed.

**Acidic medium.** Another important variable influencing the reaction between AA and MB is the acidic medium. Typically, for analytical purposes, hydrochloric acid at pH about 3 is used.[16] Nevertheless, in the proposed methodology the acid is also critical for sample emulsification since its nature can influence the formation of radicals upon sonication due to the cavitation phenomenon. In order to test this variable, different acids were studied using AA in aqueous solution and in the presence of a cosmetic sample (emulsion). The obtained results are shown in Fig. 3 and S4.† As can be seen, there are no significant differences between kinetic curves obtained with AA in solution and in the presence of emulsion using acetic acid or formic acid (Fig. 3). However, when hydrochloric or nitric acid is used, clear differences were observed (Fig. S4†). This parameter is key when considering external calibration with aqueous standards. Since acetic acid is the one providing the smallest difference in the slope for the linear relationship Log(Abs) *vs.* time (min) with and without the sample matrix, it was selected for further studies.

**Type of surfactant or stabilizer.** As mentioned above, the use of a surfactant or stabilizer is essential for the emulsification of the cosmetic sample, besides influencing the reaction rate. In general, surfactants may interact with the reactants catalysing or inhibiting the reaction through changes in the dielectric constant and/or the molecule charge.[22] In the case of stabilizers, they can modify the diffusivity, and in the case of PEG, it is also considered as a possible catalyst in organic reactions.[23] In order

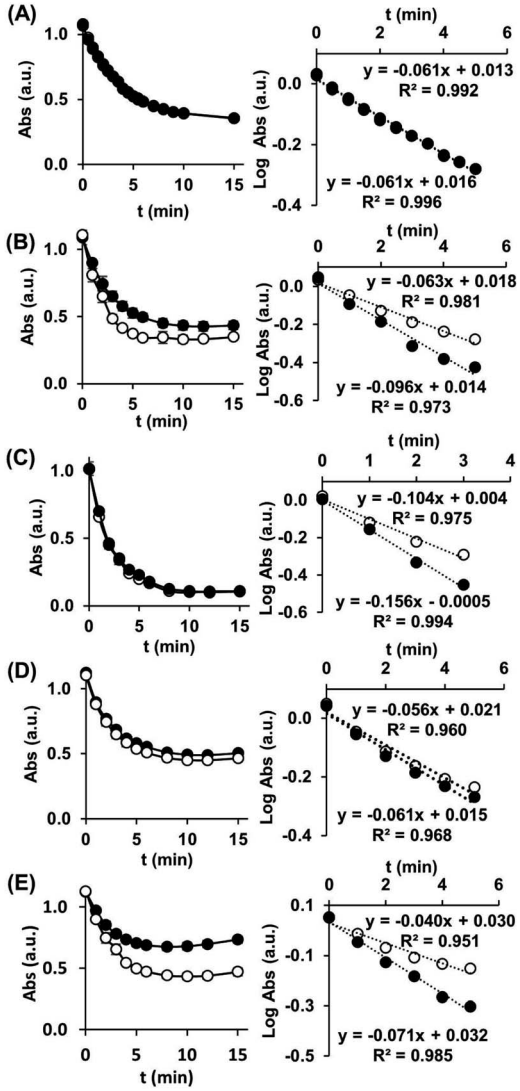


Fig. 4 Kinetic curves and graphical representation of Log(Abs) *vs.* *t* (min) with (•) and without (o) emulsion using different surfactants: (A) SDS; (B) PVP; (C) PEG; (D) Triton X-100; (E) Tween-80 (0.04 mM MB; 0.5% m/v of surfactant; 1% v/v acetic acid; 2 μg $g^{-1}$ AA).

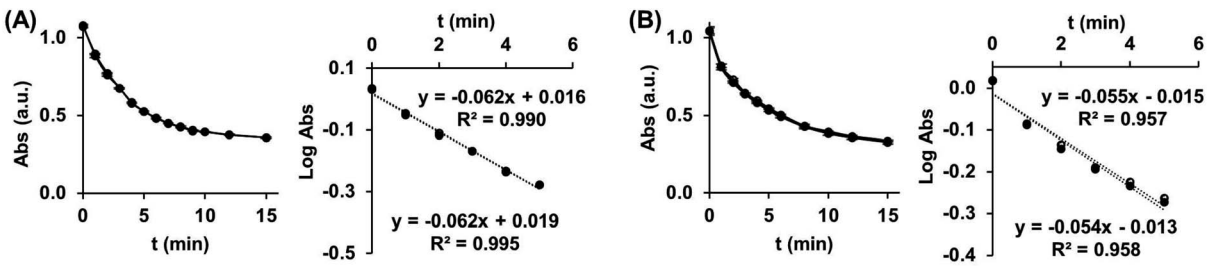


Fig. 3 Kinetic curves and graphical representation of Log(Abs) *vs.* *t* (min) with (•) and without (o) emulsion for (A) acetic acid and (B) formic acid (0.04 mM MB; 0.5% m/v SDS; 1% v/v of acid; 2 μg $g^{-1}$ AA).

 

to evaluate the influence of this parameter, kinetic curves were obtained with SDS, Triton X-100, Tween 80, PEG and PVP using AA in solution and in emulsion. As can be seen in Fig. 4, most surfactants or stabilizers modify the kinetic curves resulting in significant differences between the slopes of the linear relationship of Log(Abs) *vs.* time (min).

No differences were observed with SDS, so this surfactant was finally selected. Subsequently, the SDS concentration in the emulsion was studied, a concentration of 0.5% (m/v) being found as optimal (Fig. S5†).

### Selection of measurement time

Considering the characteristics of the reaction studied in this work, especially the influence of light on the reaction rate and precision, a fixed time kinetic method was developed. In view of the kinetic curves obtained for both aqueous standards and emulsions using SDS as the surfactant and acetic acid (Fig. 4(A)), a fixed time of 5 min was selected as a compromise solution, since it allows obtaining an adequate sensitivity and sample throughput.

### Interference study: AA derivatives and other common antioxidants present in cosmetics

As mentioned above, many current cosmetics with vitamin C as a commercial claim contain AA derivatives or, in some cases, mixtures thereof with pure AA. Although it is expected that these derivatives show lower antioxidant capacity than AA,[5] they can be considered potential interferents in the reaction with MB. The following AA derivatives were studied as potential interferents: 3-*O*-ethyl L-ascorbic acid (EAA), L-ascorbyl palmitate (AP), L-ascorbyl 2-*O*-α-glycoside (AG) and sodium L-ascorbyl phosphate (SAP). These AA derivatives were selected as the most used in facial cosmetics. Both aqueous solutions and emulsions of these AA derivatives were prepared with and without different surfactants and stabilizers. All solutions were treated following the approach described in the Measurement procedure section. The obtained results are shown in Fig. 5(A) and S6(A).† In no case, the initial absorbance of MB (Abs ~1.0) was modified in the presence of the tested AA derivates. Therefore, under the conditions used, these compounds did not interfere with the determination of AA. On the other hand, cosmetics often contain other antioxidants such as α-tocopherol, citric acid and/or salicylic acid.

Hence, these compounds were also studied as potential interferents at different concentrations. The tested concentrations were established considering the maximum allowable concentration and/or the usual ranges for each antioxidant in cosmetic matrices. The maximum allowed concentrations of α-tocopherol and salicylic acid are 5% m $m^{-1}$ and 2% m $m^{-1}$, respectively. Citric acid is usually found at concentrations in the range of 0.1–0.5% m $m^{-1}$ in cosmetics.[22]

None of these antioxidants caused significant changes in the absorbance signal of MB (Fig. S6(B)†), which implies that they do not interfere under the conditions used in this work. In addition, the possible influence of these antioxidants in the presence of AA (2 μg $g^{-1}$) was also studied. As shown in Fig. 5(B), the decrease in the initial absorbance of MB depends only on the amount of AA present in the sample.

### Analytical characteristics

Analytical characteristics for the proposed method are shown in Table 1. Calibration was carried out with aqueous standards using Log(Abs) at 5 min *vs.* the AA concentration expressed as μg $mL^{-1}$ (Fig. S7†). The limit of detection (LOD) and limit of quantification (LOQ) were calculated following the $3\sigma$ and $10\sigma$ criteria, respectively. Instrumental and procedural limits were calculated. Repeatability and reproducibility (between days) values, expressed as relative standard deviation (RSD, %), were obtained at two levels of AA in the emulsion, *i.e.*, 2 and 5 μg $g^{-1}$. To evaluate the accuracy of the developed method, recovery studies at two levels were carried out with different spiked water-based cosmetic samples of variable composition and texture, which do not contain AA in their ingredients (Table S1†). The obtained results are shown in Table 2, recovery values being in the range of 97.5–100.7%.

A comparison of the proposed approach with other reported procedures for the determination of AA in cosmetic samples is shown in Table 3. As can be observed, high performance liquid chromatography (HPLC) coupled to UV-vis is by far the most widely used detection technique for AA analysis in cosmetics.

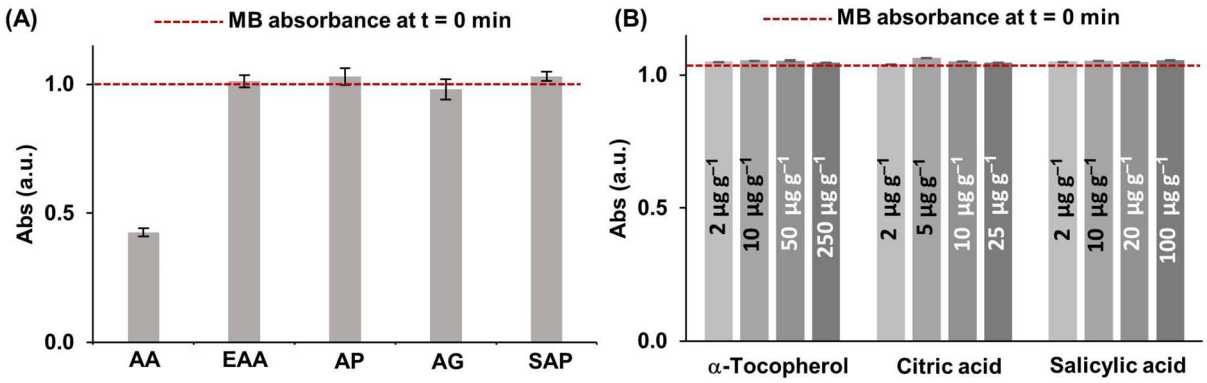


Fig. 5 (A) Analytical response of AA and AA derivates; (B) analytical response of AA in the presence of other antioxidants at different concentrations (0.04 mM MB; 0.5% m/v of SDS; 1% v/v acetic acid; irradiation time under a W lamp: 5 min).

Table 1 Analytical figures of merit of the proposed methodology for AA analysis in cosmetic samples

| Parameter | Value | |
|---|---|---|
| Calibration curve (μg mL$^{-1}$) | $y = -0.1422\ (\pm 0.0004)x + 0.1105\ (\pm 0.0013)$ | |
| $R^2$ | 09 980 | |
| Linear range (μg mL$^{-1}$) | LOQ – 7 | |
| LOD | 0.4 μg mL$^{-1}$ | 8 μg g$^{-1}$ |
| LOQ | 0.15 μg mL$^{-1}$ | 30 μg g$^{-1}$ |
| Repeatability (RSD, %) ($n = 3$) | 0.6 (2 μg g$^{-1}$) | 0.4 (5 μg g$^{-1}$) |
| Reproducibility (RSD, %) ($n = 3$) | 1.5 (2 μg g$^{-1}$) | 0.8 (5 μg g$^{-1}$) |

Regarding the sample preparation step, one of the main advantages of our approach as compared to chromatographic methods is the absence of a centrifugation and/or filtration step before measurement. The obtained emulsion can be directly used to perform the reaction with MB. Emulsification, colorimetric reaction/measurement can be accomplished within only 8 min (3 min for emulsification + 5 min for reaction with MB). Furthermore, using a cup-horn sonoreactor for the emulsification step, up to 6 samples can be simultaneously treated, thus achieving a high sample throughput. Comparing the LODs, the value obtained in the present work is better than most of those reported by HPLC-UV-vis. Besides, in comparison to HPLC coupled to chemiluminescence, the LOD obtained with the proposed approach is around 7 times better. In the case of micellar electrokinetic chromatography (MEKC), the proposed method shows around 200 times higher LOD. Besides, the use of a kinetic method offers high selectivity, reducing interference from other species being useful to solve mixtures of related compounds.

### Application of the proposed method to cosmetic samples with AA

Finally, three cosmetic samples containing pure AA in their composition were analysed for different days. The selected samples were: (i) ISDIN "Flavo-C Forte" serum with 15% pure AA and a stability of 10 days according to the product specifications; (ii) La Roche-Posay "pure vitamin C10" serum with 10% pure AA and stable for 3 months according to the product specifications; (iii) Nacomi "light it up" serum with 10% pure AA, and stable for 3 months according to the product specifications. As shown in Table 4, the initial AA value obtained and that declared by the manufacturer were comparable in all cases. No significant differences occurred between the certified and found values when applying a *t*-test ($\alpha = 0.05$).

For performing AA stability studies in the different formulations, all serum samples were stored at 6 °C and protected from light, as recommended for this type of cosmetic product to ensure good stability. The results are shown in Fig. 6. In the case of the ISDIN serum sample, the AA was stable for 15 days, 5 days longer than those guaranteed by the manufacturer. Besides, regarding the La Roche-Posay serum sample, the formulation was stable up to 105 days (15 weeks), which entails a stability 3 weeks longer than that indicated by the manufacturer. Finally, the results obtained for the Nacomi serum sample showed that the AA was stable in the formulation for 98 days (14 weeks), *i.e.*, 2 weeks longer than the stability indicated in the product specifications.

Table 2 Results for recovery studies of AA in different cosmetic samples

| Sample | Spiked value (μg g$^{-1}$) | Found value (μg g$^{-1}$ ± *s*, $N = 3$) | Recovery (% ± *s*, $N = 3$) |
|---|---|---|---|
| Armonia® facial serum | 2 | 2.01 ± 0.01 | 100.5 ± 0.6 |
| | 5 | 4.87 ± 0.04 | 97.5 ± 0.7 |
| Babaria® moisturizing oil free facial cream | 2 | 1.99 ± 0.01 | 99.7 ± 0.7 |
| | 5 | 5.02 ± 0.04 | 100.4 ± 0.8 |
| Clarins® HydraQuench facial cream | 2 | 2.00 ± 0.01 | 100.1 ± 0.4 |
| | 5 | 4.97 ± 0.01 | 99.5 ± 0.3 |
| Elizabeth Arden® ceramide capsules | 2 | 1.99 ± 0.01 | 99.5 ± 0.7 |
| | 5 | 4.93 ± 0.01 | 98.6 ± 0.3 |
| Elizabeth Arden® ceramide night cream | 2 | 1.97 ± 0.01 | 98.4 ± 0.4 |
| | 5 | 5.00 ± 0.04 | 100.7 ± 0.8 |
| Estée Lauder® future perfect anti-wrinkle eye cream | 2 | 2.00 ± 0.03 | 100.4 ± 0.3 |
| | 5 | 4.90 ± 0.03 | 98.0 ± 0.5 |
| Farmacia Gel® moisturizing facial cream | 2 | 2.01 ± 0.01 | 100.5 ± 0.6 |
| | 5 | 4.95 ± 0.04 | 98.9 ± 0.7 |
| L'oréal Paris® revitalift filler facial serum | 2 | 1.98 ± 0.01 | 98.9 ± 0.2 |
| | 5 | 4.98 ± 0.02 | 99.7 ± 0.5 |
| NeoStrata® glycolic renewal facial serum | 2 | 1.97 ± 0.03 | 98.0 ± 0.4 |
| | 5 | 4.98 ± 0.03 | 99.7 ± 0.6 |
| Noviderm Sérénactiv® moisturizing facial emulsion | 2 | 1.96 ± 0.01 | 98.2 ± 0.4 |
| | 5 | 4.93 ± 0.04 | 98.6 ± 0.8 |
| Yves Rocher ADN Végétal® moisturizing facial cream | 2 | 1.97 ± 0.01 | 98.4 ± 0.4 |
| | 5 | 4.90 ± 0.01 | 98.0 ± 0.3 |
| Yves Rocher Sèrum Végétal® anti-ageing night cream | 2 | 1.99 ± 0.01 | 99.6 ± 0.7 |
| | 5 | 4.96 ± 0.04 | 99.2 ± 0.8 |

Table 3 Comparison of some figures of merits for the determination AA in cosmetics by different methods[a]

| Sample | Sample preparation remarks | Technique | LOD (μg mL$^{-1}$) | Linear range (μg mL$^{-1}$) | RSD (%) | Ref. |
|---|---|---|---|---|---|---|
| Intensive serum ampoules | US-assisted extraction (30 min) in phosphate buffer (pH 2.3) and methanol 10% v/v. Centrifugation (30 min) and filtration with a PTFE syringe filter | HPLC-UV-vis | 0.1 | 0.3–500 | 3.4 | 6 |
| Moisturizing water-based cream and oil/water cream | Sample extraction in a metaphosphoric acid 0.2% (v/v) : methanol : acetonitrile (90 : 8 : 2 v/v/v) mixture. Centrifugation (5 min) | HPLC-UV-vis | 0.05 | 1–12 | 1.2 | 7 |
| Whitening cream | US-assisted extraction (10 min) in phosphate buffer (pH 3) : ethanol (65 : 35 v/v). Filtration with a nylon membrane filter | HPLC-UV-vis | 5 | 15–175 | 0.6 | 8 |
| Whitening cream | US-assisted extraction (15 min) in water. Sample dilution, filtration, and deoxygenation under a $N_2$ stream (3 min) | HPLC-UV-vis | n.d | 10–300 | 3.5 | 9 |
| Whitening cream | Sample extraction in methanol. Centrifugation (15 min) and filtration with a nylon syringe filter | HPLC-CL | 0.3 | 1–200 | 4.5 | 10 |
| Moisturizing facial serum | Sample extraction in EDTA 0.05% m/v (2 min). Centrifugation (10 min) and filtration with a PTFE syringe filter | UHPLC-MS/MS | 0.0003 | 0.001–1.7 | 2.2 | 11 |
| Moisturizing facial serum | US-assisted extraction (10 min) chloroform : methanol (4 : 6 v/v). Centrifugation | MECK | 8.8 | 25–175 | 3 | 12 |
| Facial serum | US-assisted emulsification (3 min) in SDS (1% m/v) and acetic acid (2% v/v) | UV-vis | 0.04 | 0.15–7 | 0.4 | This work |

[a] US: ultrasound; PTFE: polytetrafluoroethylene; EDTA: ethylenediaminetetraacetic acid; SDS: sodium dodecyl sulphate; HPLC-UV-vis: high performance liquid chromatography coupled to UV-vis detection; HPLC-CL: high performance liquid chromatography coupled to chemiluminescence detection; UHPLC-MS/MS: ultra performance liquid chromatography coupled to tandem mass spectrometry; MECK: micellar electrokinetic chromatography.

Table 4 Analytical results for the analysis of AA in commercial cosmetic samples

| Sample | AA content[a] (%) | Found value-day 1 (% m m$^{-1}$ ± $s$, $n$ = 3) | Recovery (% ± $s$, $n$ = 3) |
|---|---|---|---|
| ISDIN® Flavo-C Forte | 15 | 14.9 ± 0.2 | 99 ± 0.8 |
| La-Roche Posay® pure vitamin C10 | 10 | 9.9 ± 0.1 | 99 ± 0.4 |
| Nacomi® light it up | 10 | 9.9 ± 0.1 | 99 ± 1.3 |

[a] Pure AA content as indicated by the manufacturer.

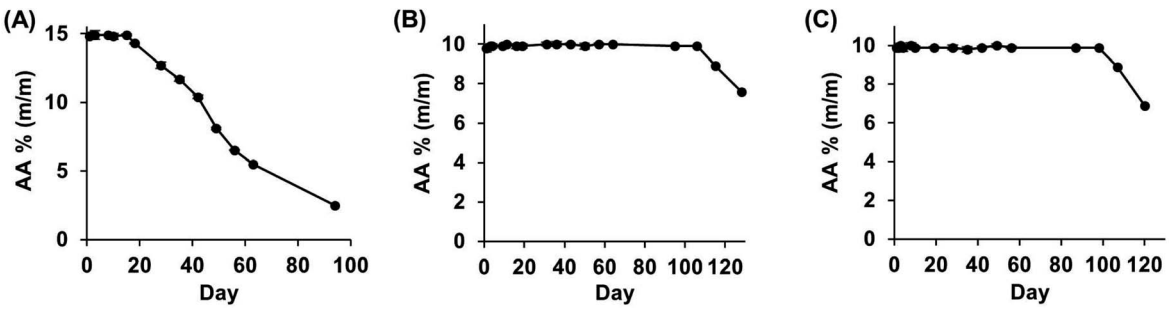


Fig. 6 Monitoring of AA in the tested facial serums: (A) ISDIN Flavo-C Forte 15% AA, (B) La Roche-Posay Pure Vitamin C10 10% AA, and (C) NACOMI 'Light it up' 10% AA.

## Conclusions

The proposed end-point kinetic method based on the ultrasound-assisted emulsification of cosmetic samples followed by a reaction with MB has proved suitable for the determination of AA in this type of complex matrix by UV-vis molecular absorption spectrophotometry. The kinetic strategy eliminates possible interference from both other antioxidants found in cosmetics and AA derivatives commonly used as substitutes of pure AA in cosmetic formulations.

Cosmetic matrix emulsification was crucial to obtain transparent and stable solutions, which can be directly used for the reaction with MB without further treatment. Considering the instability of the analyte, mild emulsification conditions (*i.e.*, time, amplitude and medium) were established to avoid the oxidation of AA. Furthermore, the use of SDS and acetic acid as reaction media both for emulsification and reaction with MB allowed the calibration with aqueous standards. In addition, the use of a closed system for irradiation under a tungsten lamp significantly improved the obtained precision (RSD 0.3%) in comparison with irradiation under sunlight (open system). Emulsification and colorimetric reaction/measurement can be accomplished within only 8 min allowing the analysis of AA in different cosmetics in a wide concentration range, from ppm to 20% m m$^{-1}$. Furthermore, with the proposed procedure, up to 6 samples can be simultaneously treated offering a high sample throughput. Neither centrifugation nor filtration is required before reaction with MB or measurement, providing a simple, rapid, sensitive, and cost-effective approach for the determination and monitoring of AA in cosmetic samples, which can be adapted to routine laboratories.

## Author contributions

Isela Lavilla: conceptualization, data curation, and writing – original draft preparation; Vanesa Romero: investigation, methodology, data curation, and writing – reviewing and editing; Paula Costas: investigation and methodology; Carlos Bendicho: supervision and writing – reviewing and editing.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This article is based upon work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society. This work was supported by the Spanish Ministry of Science, Innovation and Universities (project number RTI2018-093697-B-I00); Xunta de Galicia within the I2C postdoctoral program [grant number ED481D-2021-021].

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