Analytica Chimica Acta 659 (2010) 159–166

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Development of HPLC and UV spectrophotometric methods for the determination of ascorbic acid using hydroxypropyl-β-cyclodextrin and triethanolamine as photostabilizing agents

Claudia Garnero, Marcela Longhi*

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina

ARTICLE INFO

Article history:
Received 28 August 2009
Received in revised form
10 November 2009
Accepted 16 November 2009
Available online 20 November 2009

Keywords:
Ascorbic acid
Complexation
Photostabilization
UV spectrophotometry
High performance liquid chromatography
Validation

ABSTRACT

In this study, the effect of complex formation with triethanolamine (TEA) alone and in combination with hydroxypropyl-β-cyclodextrin (HP-β-CD) on the photostability of ascorbic acid was evaluated for exposure to artificial and diffuse daylight. The first-order rate constants for the photodegradation reactions were determined. The data obtained showed that these complexes strongly reduced the photodegradation process with an 11- and 35-fold increase in the photostability of ascorbic acid, depending of the ligand concentration and the irradiation source. The multicomponent complex gave a significantly better stabilization for exposure to light than TEA alone.

Due to the fact that the complexation extended the exposure of ascorbic acid to light (without molecular changes), UV spectrophotometric and reversed phase high performance liquid chromatographic (HPLC) methods were developed for the quantitative determination of the vitamin in pure form and in pharmaceutical preparations. These methods were statistically validated, all the validation parameters were found to be within the acceptance range. These results demonstrate that the proposed methods are suitable for the quality control of ascorbic acid, providing simple, rapid, precise, accurate and convenient approaches for routine analysis of bulk drug and pharmaceutical formulations.

1. Introduction

The photostability of drugs represents an emerging topic in the pharmaceutical research field, with the number of drugs which have been revealed to be light sensitive noticeably increasing. The photochemical behavior of a drug molecule is strongly dependent on the microenvironment, and its photoreactivity can therefore be altered by interactions with macromolecules [1]. New technology-based pharmaceutical systems have been proposed in order to enhance stability for such drugs. Some of these approaches have used chemical complexes of drugs with appropriate photoprotective carriers [2–4].

A wide area of supramolecular chemistry focuses on the host–guest complexes formed by the binding of a substrate to molecular receptors via non-covalent interactions [5–7]. In the present work, cyclodextrins (CDs) were used, which are cyclic oligosaccharides with hydroxyl groups on the outer surface and a void cavity in the center. Their outer surface is hydrophilic, but the cavity has a lipophilic character. CD encapsulation of a guest molecule affects many of its physicochemical properties. Naturally occurring CDs and their synthetic derivatives improve certain properties of the drugs, such as solubility, stability, and/or bioavailability [8]. Moreover, in our recent works it was shown that the addition of a suitable auxiliary substance, such as triethanolamine (TEA), can enhance the power of hydroxypropyl-β-cyclodextrin (HP-β-CD) as a result of a combined effect of salt formation and inclusion complexation [9,10].

This current study focuses on ascorbic acid, shown in Fig. 1, due to the fact that it is an important water soluble vitamin. It is highly sensitive to heat, alkali, oxygen and light, and also to contact with traces of copper and iron [11,12]. Although ascorbic acid has been extensively studied in different fields, interest in this vitamin has never waned and further aspects are currently being investigated. Previously, we have studied the influence of complexation with HP-β-CD and TEA on the degradation rate of ascorbic acid in solution. The results obtained showed a pronounced enhancement of aqueous stability with the TEA association complex, whereas the HP-β-CD inclusion complex had a minor effect. In addition, the multicomponent complex produced a significantly better stabilization than that of HP-β-CD alone [13].

The degradation of ascorbic acid in aqueous solutions, however, was found to be very sensitive to laboratory fluorescent lighting exposure. This photochemical behavior has important analytical implications and clearly complicates the assays. The aim of the

* Corresponding author. Tel.: +54 351 4334163; fax: +54 351 4334127.
E-mail address: mrlcor@fcq.unc.edu.ar (M. Longhi).

0003-2670/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2009.11.038

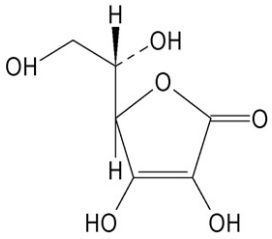

Fig. 1. Chemical structure of ascorbic acid.

present study was to find a suitable photostabilizer complex and to determine its optimum concentration for the analysis of this vitamin. We studied the potential of TEA and the TEA:HP-β-CD combination to reduce the photodecomposition of ascorbic acid, under laboratory fluorescent lighting and simulated sunlight irradiation.

Currently, some difficulties still remain to quantify ascorbic acid due to its extreme instablility in aqueous solutions, especially upon exposure to light. Several analytical methods including chromatography [11,14,15], microcalorimetry [16], fluorimetry [17], electrochemistry [18,19], and spectrophotometry [20–22], have been tried in different pharmaceutical forms, either alone or in combination with other drugs, as well as in foods or biological materials among others. Nevertheless, the majority of these require previous sample preparation steps, such as derivatization or elimination of matrix effects which often makes the method more complicated and laborious. Besides, these methods often do not take special precautions to prevent degradation of the drug.

In the present study, UV spectrophotometric and HPLC methods were developed and validated for ascorbic acid that were based on the significant enhancement of its stability in aqueous solutions and of its photostability due to the formation of complexes. These methods were successfully used to assay the total content of ascorbic acid in different pharmaceutical formulations.

2. Experimental

2.1. Chemicals and equipment

Ascorbic acid was obtained from Anedra® (99%) (Buenos Aires, Argentina) and TEA was purchased from Aldrich® (98%) (Milwaukee, WI, USA). HP-β-CD (MW = 1326–1400, degree of molar substitution 7.0) was kindly supplied by the Ferromet (Buenos Aires, Argentina) subsidiaria of Roquette (Lestrem, France). HPLC grade methanol was procured from Sintorgan (Buenos Aires, Argentina). All experiments were performed using water purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other materials and solvents were of analytical reagent grade.

A Shimadzu UV-160A spectrometer (VA Howe, UK) with 10 mm quartz cells was used for all spectral measurements.

The HPLC system consisted of an Agilent 1100 series pump, an autosampler, a multiple-wavelength ultraviolet–visible (UV–vis) detector and a Chemstation software version A.10.02 (Agilent, Waldbronn, Germany). The column used was a Phenomenex Gemini C18 250 mm × 4.6 mm i.d. filled with 5 μm particles, with a precolumn (guard cartridge SecurityGuard C18 4 mm × 3.0 mm i.d.) supplied by Phenomenex (Torrance, CA, USA).

2.2. Photostability studies

The kinetics of photodegradation of ascorbic acid were studied in solution in the presence or absence of TEA alone and in the combination of TEA and HP-β-CD. The effect of different concentrations of TEA was evaluated, while for the combination TEA:HP-β-CD, the concentration of HP-β-CD was kept constant.

In addition, the influence of both pH and light on the photodegradation was studied at different pH values. The studies were performed using 50 mM $NaHCO_3$ adjusted with NaOH or phosphoric acid to different pH values, and McIlvaine buffers prepared from 0.2 M Na_2HPO_4 and 0.1 M citric acid.

Stock solutions of ascorbic acid (0.5 mg mL^{-1}) were prepared in water and protected from light before irradiation. Freshly prepared solutions were used for each experiment in order to avoid any chemical or photochemical effects. Test solutions were prepared by diluting the stock solutions to a final concentration of 1.8×10^{-2} mg mL^{-1} in water and buffer solutions. The aqueous solutions containing increasing concentrations of TEA (0.9454–3.5450 mM) or their combination with HP-β-CD (0.9454–1.7365 mM and 1.00% (w/v), respectively). The samples were exposed to light sources in cylindrical tubes of transparent glass (13 mm i.d. × 100 mm) under continuous stirring, and maintained in a water bath at constant temperature of 25.0 ± 0.1 °C [Haake DC10 thermostat (Haake, Paramus, NJ, USA)] to minimize the degradation produced by effect of thermal reactions. One set of these solutions was irradiated with a Philips mercury arc lamp (emission in the range of 312–577 nm) fixed horizontally at a distance of 50 cm which transmitted light corresponding to exposure behind a glass window. The other set was positioned 160 cm away from daylight fluorescent tubes (Philips, TLT 40W/54), fixed horizontally (emission in the range of 400–600 nm). At specified time intervals, samples were withdrawn and immediately analyzed for remaining ascorbic acid by spectrophotometrically monitoring the decrease in absorbance at 266 nm. Each experiment was performed in triplicate.

2.3. Preparation of stock and standard solutions for proposed methods

Stock solutions of ascorbic acid (0.29 mg mL^{-1}) were prepared in water and protected from light. The standard solutions were prepared by dilution of appropriate aliquots of these stock solutions with TEA 0.9749 mM solution and TEA 0.9749 mM:HP-β-CD at 1.00% (w/v) solution, respectively.

2.4. Conditions of proposed methods

Spectroscopic determinations were carried out at room temperature. The absorbance was measured for each system at 266 nm against a reagent blank prepared under identical conditions without addition of the examined drug.

HPLC experiments were done using isocratic conditions. The mobile phase was filtered through a 0.45 μm Millipore membrane and degassed prior to use. The column temperature was 25 °C, and the injection volume was 50 μL. The assay procedure was performed using the external standard method.

2.5. Validation of proposed methods

The developed methods were validated according to standard procedures (ICH Guidelines, 2005 [23]).

Linearity and range were studied by preparing the calibration curves, which were constructed in triplicates at seven concentration levels. Additionally, linearity of the calibration graphs and conformity with the Lambert–Beer Law for the systems were evaluated by the *F*-test.

The detection (LOD) and the quantification (LOQ) limits were calculated based on the standard deviation (SD) and the slope of the calibration graphs.

Similarly, precision and accuracy were determined by analyzing three different concentrations of the acid in triplicate. The precision was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentrations in triplicate on the same day. Accuracy was determined for both intra- and inter-day variations by analyzing the samples in triplicate.

2.5.1. Spectrophotometric method

Calibration curves were ranging from 12.0 to 20.4 μg mL^{-1} for dilution with TEA solution, or TEA:HP-β-CD solution. Intermediate precision was checked by repeating the studies on four different days.

2.5.2. Chromatographic method

The calibration curves were prepared in the concentration range of 12.2–21.4 μg mL^{-1} for dilution with TEA solution or TEA:HP-β-CD solution. Intermediate precision was checked by repeating the studies on five different days. All solutions were injected in triplicate. Moreover, six replicates of a standard solution were analyzed to assess the system precision.

Additionally, calibration curves ranging from 12.5 to 20.8 μg mL^{-1} were prepared in water. These solutions were analyzed immediately after preparation in order to avoid degradation of ascorbic acid.

2.6. Titration method

Volumetric titration, based on the monograph of the USP [24] for the analysis of ascorbic acid, was used as the reference method. This method is based on the reaction of ascorbic acid with an iodine solution, using starch as an indicator for the end point of the titration.

2.7. Analysis of pharmaceutical formulations

The developed methods were applied for the analysis of ascorbic acid in different pharmaceutical formulations. For the purpose of comparison, the same samples were analyzed using the reference method. The percentage of ascorbic acid recovered in each sample was calculated by dividing the determined by the labeled amount of the drug.

Four commercially available formulations of ascorbic acid were selected to estimate the total drug content by the proposed methods. Redoxon® Drops (Roche Products Q.F.S.A., Brazil), with a declared content of 200 mg mL^{-1} of ascorbic acid; Redoxon® Effervescent (Roche Products S.A.Q. e I., Argentina), labeled as containing 2 g of ascorbic acid; C-Vitamin Effervescent (Asofarma, Argentina), labeled as containing 1 g of ascorbic acid; and Tanvimil C tablets (Laboratories Raymos SAIC, Argentina), which declared a content of 500 mg of ascorbic acid.

2.7.1. Samples preparation

For drops, an aliquot volume (equivalent to 200 mg of ascorbic acid) was taken and dissolved in water. This solution was then appropriately diluted with the same solvent.

Tablets and effervescent tablets were weighed and finely powdered. An amount of powder equivalent to 200 mg of ascorbic acid was weighed and dissolved in water. The suspensions were sonicated for 10 min, with any remaining residues being removed by filtration. The clear solutions obtained were then appropriately diluted with water.

All resulting solutions were diluted with TEA or TEA:HP-β-CD solutions to obtain final concentrations within the limits of linearity for the proposed methods.

For the chromathographic method, each preparation was filtered through 0.45 μm membrane filters.

For the reference method, quantities of formulations equivalents to 150 mg of ascorbic acid were taken, dissolved in acidic medium and then titrated.

3. Results and discussion

3.1. Photostability studies

In order to investigate the influence of binary and multicomponent complexes on the photochemical degradation processes of ascorbic acid, the experiments were performed on solutions exposed to laboratory fluorescent lighting and simulated sunlight radiation. These radiation sources simulate the real conditions of exposure of the samples during analytical experiments. In each study the samples were exposed to light, according to ICH Guideline [25], on the assumption that the solutions are exposed under real conditions to a mixture of glass-filtered natural light and/or indoor light.

The degradation rate of free ascorbic acid in solution was compared to its photochemical degradation in solutions containing ligands under identical experimental conditions.

For the light stability studies, the measurement of the absorbance maximum peaks was used throughout to directly determine the remaining ascorbic acid. The presence of TEA and HP-β-CD did not cause any appreciable change in its absorption spectrum. Moreover, no significant absorption was detected for the photoproduct at this wavelength.

The ascorbic acid degradation curves, plotted in Fig. 2, were linear, indicating that the photodegradation of this drug and its complexes in aqueous solutions followed apparent first-order reactions. To determine the kinetic parameters, important from the pharmaceutical point of view, a model of the first-order reaction was used. The rate constants of photodegradation in the absence of ligands (k_0), the observed rate constants of photodegradation in the presence of ligands (k_{obs}), the half-life (t_{50}) and the time of 10% degradation of the drug (t_{90}) were estimated by linear regression analysis.

The profiles obtained using both sources were similar in shape, indicating that the wavelengths of irradiation used had no significant effect on the nature of the reaction [26]. The parameters of the photodegradation reactions carried out using simulated sunlight irradiation (mercury arc lamp) and laboratory fluorescent lighting (daylight fluorescent tubes) are shown in Tables 1 and 2, respec-

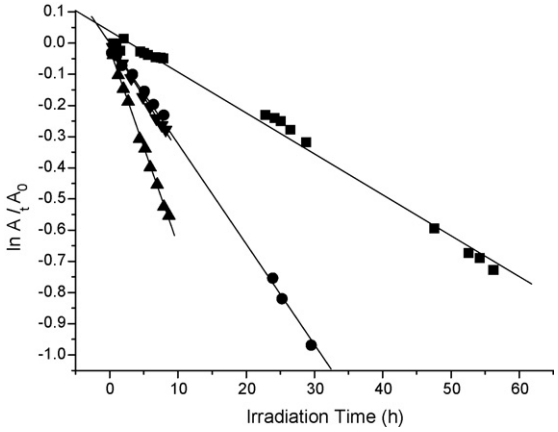

Fig. 2. Remaining percentage of ascorbic acid, after exposure to a mercury arc lamp in the presence of TEA (▲), and TEA:HP-β-CD (▼); and after exposure to daylight fluorescent tubes in the presence of TEA (■), and TEA:HP-β-CD (●).

Table 1
Effect of irradiation with a mercury arc lamp on the photostability of ascorbic acid complexes in solution.

Ligand concentration		pH	k_0 (h^{-1})	k_{obs} (h^{-1})	t_{90} (h)	t_{50} (h)	k_0/k_{obs}
TEA	0.9454 mM	7.97	0.47 ± 0.01	0.0275 ± 0.0008	3.8	25.2	17.09
	0.9749 mM	7.93		0.0238 ± 0.0002	4.4	29.1	19.75
	1.737 mM	8.41	0.58 ± 0.02	0.033 ± 0.003	3.1	20.8	17.67
	2.954 mM	8.66	0.69 ± 0.02	0.060 ± 0.004	1.8	11.6	11.45
	3.545 mM	8.66		0.055 ± 0.003	1.9	12.7	12.49
HP-β-CD 1.00% (w/v):TEA	0.9454 mM	8.15	0.49 ± 0.03	0.0137 ± 0.0001	7.7	50.7	35.91
	0.9749 mM	8.12		0.0278 ± 0.0003	3.8	24.9	17.70
	1.737 mM	8.63	0.69 ± 0.02	0.035 ± 0.002	3.0	20.0	19.63

tively.

The results reveal that the formation of a complex had a stabilizing effect on the ascorbic acid with respect to photodegradation of the free molecule in solution (using pH adjustment to produce identical experimental conditions). The free ascorbic acid was photodegraded at higher rates than in the complex form.

The evaluation of the relations k_0/k_{obs} in the presence of TEA or TEA:HP-β-CD system, showed that the complexation method retarded the photodegradation of ascorbic acid. In addition, TEA alone made ascorbic acid more stable when irradiated with laboratory fluorescent lighting than when it was exposed to simulated sunlight radiation. Comparison of the values of the k_{obs} demonstrated that ascorbic acid solutions containing the combination of TEA and HP-β-CD also produced photodegradation of the drug, but at a lower rate than the solutions containing only TEA. It therefore seems likely that TEA and HP-β-CD had a synergistic effect on the photodegradation of ascorbic acid.

3.2. Development and optimization of the methods

The results obtained appear very promising, and clearly confirm that the complexation offered a higher stability against photoinduced degradation, which normally limits the application of different analytical methods. The stabilizing effect of the complexation provided sufficient time to prepare the analytical samples and perform the subsequent determinations. This was carried out by the development of spectrophotometric and chromatographic methods.

3.2.1. Spectrophotometric method

As previously described, the UV spectrum of ascorbic acid solutions show significant bathochromic shifts of the λ_{max}, with changes in the ascorbic acid concentration, producing considerable deviations from the Lambert–Beer Law. This dramatic shift in the value of λ_{max} may be due to the chromophore group, which is very sensitive to dissociation, with even small variations in the enviromental pH giving rise to bathochromic shifts [27]. Therefore, it is clear that λ_{max} should be determined for each solution, because spectrophotometric readings for only one λ (as recommended in several works) can produce imprecise data. Nevertheless, the results obtained in our previous study demonstrated that complexation with HP-β-CD and TEA, either separately or in combination, increases not only the stability of ascorbic acid but also the value of λ_{max} [13]. An important implication of maintaining the value of λ_{max} is that it permits the development of reliable analytical methods for stability studies and quality control of pharmaceutical products containing ascorbic acid.

To develop sensitive UV methods, two complexation systems were tested, TEA alone and TEA with HP-β-CD. The absorption maximum of ascorbic acid, in both aqueous systems, was found to be 266 nm, without shift changes in the drug concentration range studied. The formation of binary and ternary complexes allowed the use of conventional UV spectrophotometry for the assay of ascorbic acid.

3.2.2. Chromatographic method

The development of the HPLC method started with the selection of the mobile phase composition and the flow rate to optimize the retention time and the tailing factor. Methanol, acetonitrile and phosphate buffer (KH_2PO_4, adjusted to different pH values) were used as the basis for the different mobile phases tested. The best mobile phase was found to be methanol–phosphate buffer (0.01 M KH_2PO_4, pH 2.0) 35:65 (v/v). The optimum flow rate was 1.5 mL min^{-1}, resulting in a maximum run time of 5 min.

The resultant chromatograms under the experimental conditions, shown in Fig. 3, showed a single peak of the ascorbic acid with a retention time of around 2.5 min in aqueous solution and around 1.9 min in presence of ligands, and a peak tailing factor within the acceptable limit (<1.5). It demonstrated that the formation of complexes with these ligands reduced the retention time of the analyte. Hence, the lower run time consumes less mobile phase solvents resulting cost-effective during the routine analysis of drug samples.

An analytical wavelength of 245 nm was chosen, since the absorbance maximum for ascorbic acid in the mobile phase occurs at this wavelength.

3.3. Validation of the developed methods

The specificity of the analytical methods was established by analyzing samples which were subjected to stress conditions. For thermal degradation of ascorbic acid, aqueous solutions were

Table 2
Effect of irradiation with daylight fluorescent tubes on the photostability of ascorbic acid complexes in solution.

Ligand concentration		pH	k_0 (h^{-1})	k_{obs} (h^{-1})	t_{90} (h)	t_{50} (h)	k_0/k_{obs}
TEA	0.9454 mM	7.97	0.41 ± 0.03	0.0193 ± 0.0008	5.4	35.8	21.24
	0.9749 mM	7.93		0.0169 ± 0.0005	6.2	40.8	24.26
	1.737 mM	8.41	0.47 ± 0.03	0.0140 ± 0.0005	7.5	49.3	33.57
	2.954 mM	8.66	0.51 ± 0.02	0.028 ± 0.005	3.8	25.0	18.36
	3.545 mM	8.66		0.0238 ± 0.0002	4.4	29.1	21.60
HP-β-CD 1.00% (w/v):TEA	0.9454 mM	8.15	0.43 ± 0.03	0.0142 ± 0.0001	7.4	48.7	30.21
	0.9749 mM	8.12		0.0159 ± 0.0004	6.6	43.7	26.98
	1.737 mM	8.63	0.51 ± 0.02	0.032 ± 0.002	3.3	21.9	16.06

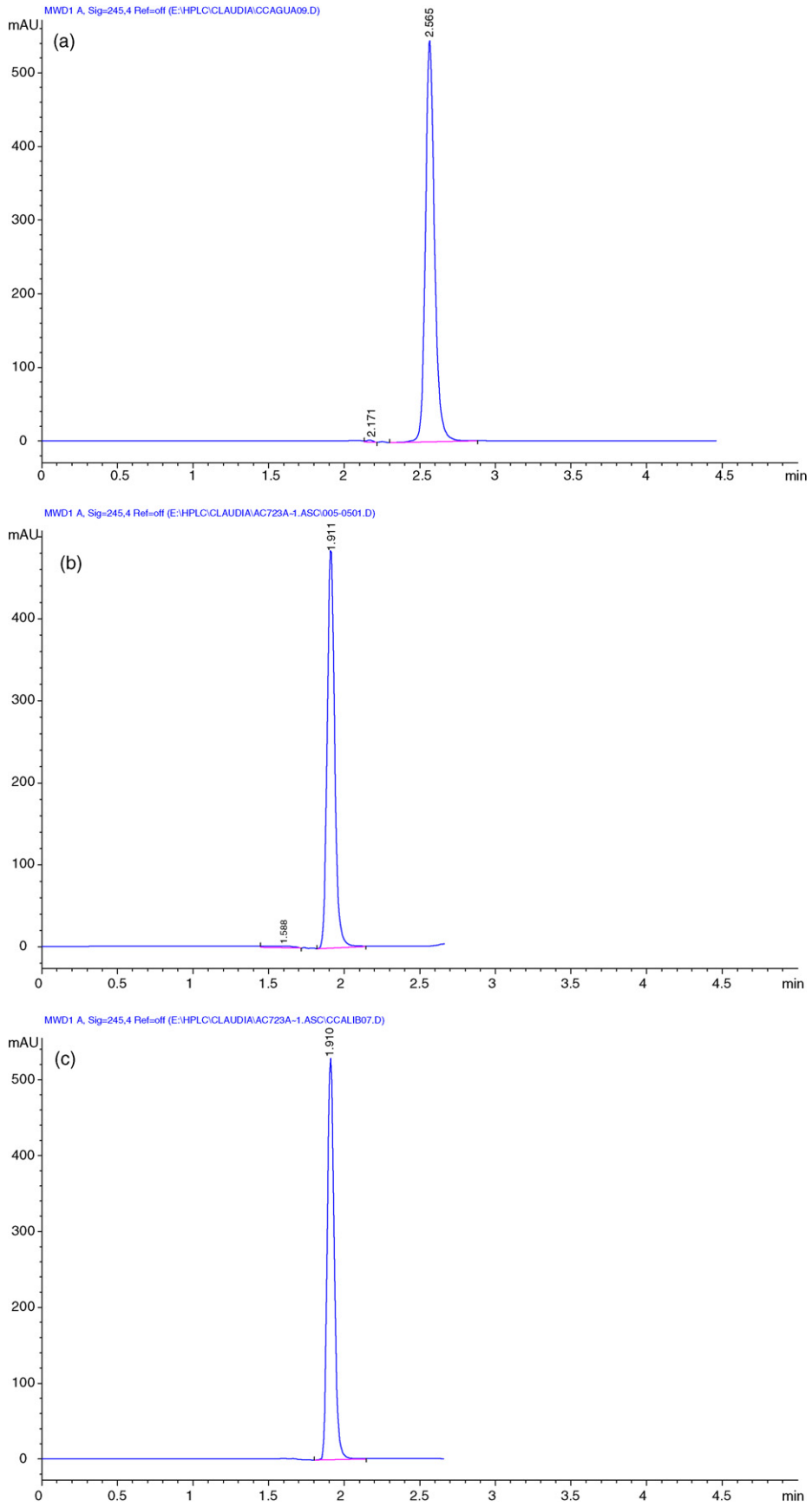

Fig. 3. Chromatogram of ascorbic acid corresponding to experiments: (a) in aqueous solution, (b) in TEA 0.9749 mM solution, and (c) in TEA 0.9749 mM:HP-β-CD 1.00% (w/v) solution.

Table 3
Analytical figures of merit of the proposed methods.

Analytical parameter	Results			
	Spectrophotometric method		Chromatographic method	
	TEA 0.9749 mM	TEA 0.9749 mM:HP-β-CD 1.00% (w/v)	TEA 0.9749 mM	TEA 0.9749 mM:HP-β-CD 1.00% (w/v)
Linear range ($\mu g\,mL^{-1}$)	12.0–20.4		12.2–21.4	
Slope (b)	0.079	0.083	106.44	83.18
Intercept (a)	0.16	0.02	73.356	132.6
SD of slope (S_b)	0.001	0.001	1.23	1.49
SD of intercept (S_a)	0.02	0.02	21.004	25.7
LOD ($\mu g\,mL^{-1}$)	1.00	0.73	0.77	0.91
LOQ ($\mu g\,mL^{-1}$)	2.89	2.10	2.20	2.77

Table 4
Validation report for determination of ascorbic acid in standard solutions by spectrophotometric methods.

TEA 0.9749 mM				TEA 0.9749 mM:HP-β-CD 1.00% (w/v)			
Nominal concentration ($\mu g\,mL^{-1}$)	Measured concentration ($\mu g\,mL^{-1}$) ± SD	Accuracy (% recovery)	Precision (% R.S.D.)	Nominal concentration ($\mu g\,mL^{-1}$)	Measured concentration ($\mu g\,mL^{-1}$) ± SD	Accuracy (% recovery)	Precision (% R.S.D.)
Intra-day (repeatability)							
13.6	13.5 ± 0.1	99.3	0.98	12.9	12.9 ± 0.1	100.0	0.80
16.3	16.4 ± 0.1	100.6	0.75	16.2	16.18 ± 0.09	99.9	0.59
20.4	20.3 ± 0.1	99.5	0.69	20.5	20.5 ± 0.1	100.0	0.53
Inter-day (intermediate precision)							
13.6	13.6 ± 0.2	100.0	1.21	12.9	13.1 ± 0.1	101.5	0.80
16.3	16.3 ± 0.1	100.0	0.95	16.2	16.2 ± 0.2	100.0	1.10
20.4	20.4 ± 0.3	100.0	1.55	20.5	20.4 ± 0.2	99.5	1.15

placed at 60 °C for 3 h, then cooled to room temperature and analyzed; similarly, for photolytic degradation, drug solutions in water were exposed to light for 72 h, and then analyzed. Thermal and photolitic degradation products of ascorbic acid aqueous solutions, did not show significant absorbance in the absorption spectra. Therefore, these methods were specific, since ascorbic acid could be determined without any interference from degradation products.

3.3.1. Spectrophotometric method

For the experimental conditions described previously, linear regression equations were established from the calibration. Table 3 contains the figures of merit of the proposed methods. The ascorbic acid concentration showed a linear relationship between the absorbance at 266 nm and the concentration range for both systems. Moreover, the calculated F-values did not exceed the tabulated values at 5% significance levels, proving the linearity of the calibration graphs and the conformity with Lambert–Beer law for both systems.

Table 4 provides data obtained from the recovery studies. The repeatability, intermediate precision and accuracy were evaluated by preparing three samples at each concentration. The relative standard deviation (R.S.D.) values for repeatability and the intermediate precision studies were found to be within the acceptable limits of less than 2.0% for the two systems. These results confirm that the methods were sufficiently precise. Excellent recoveries of ascorbic acid were obtained at each concentration. These results were within 100 ± 2%, indicating that the proposed methods were accurate.

3.3.2. Chromatographic method

For the described experimental conditions, the figures of merit of the method were established and are listed in Table 3. A linear correlation was obtained between the peak area ratio and ascorbic acid concentration over the concentration range. Additionally, a calculated F-value less than the tabulated value at 5% significance level, proved the linearity of calibration graphs.

Results for system precision test performed on each of the standard solution of ascorbic acid showed that the % R.S.D. was 0.73 in presence of TEA alone and 0.87 for the TEA:HP-β-CD combination. Therefore, the system precisions are considered to be satisfactory.

The repeatability, intermediate precision and accuracy were evaluated by the recovery studies. Three samples were prepared at each concentration. The results of experiments are shown in Table 5.

Table 5
Validation report for determination of ascorbic acid in standard solutions by chromatographic method.

Aqueous solution		TEA 0.9749 mM				TEA 0.9749 mM:HP-β-CD 1.00% (w/v)			
Nominal concentration ($\mu g\,mL^{-1}$)	Measured concentration ($\mu g\,mL^{-1}$) ± SD[a], recovery (%), R.S.D. (%)	Nominal concentration ($\mu g\,mL^{-1}$)	Measured concentration ($\mu g\,mL^{-1}$) ± SD	Accuracy (% recovery)	Precision (% R.S.D.)	Nominal concentration ($\mu g\,mL^{-1}$)	Measured concentration ($\mu g\,mL^{-1}$) ± SD	Accuracy (% recovery)	Precision (% R.S.D.)
Intra-day (repeatability)									
12.5	11.2 ± 1.1, 89.6, 9.75	12.5	12.465 ± 0.007	99.7	0.06	12.5	12.5 ± 0.1	100.0	0.88
15.8	11.6 ± 0.9, 73.4, 8.18	15.8	15.9 ± 0.1	100.6	0.89	15.8	15.8 ± 0.2	100.0	1.02
18.9	10.8 ± 1.2, 57.2, 10.91	18.9	18.9 ± 0.2	100.0	1.05	19.2	19.2 ± 0.3	100.0	1.35
Inter-day (intermediate precision)									
		12.5	12.4 ± 0.1	99.2	0.97	12.5	12.6 ± 0.2	100.8	1.50
		15.8	15.8 ± 0.2	100.0	1.04	15.8	15.8 ± 0.2	100.0	1.23
		18.9	18.7 ± 0.2	99.0	1.15	19.2	19.3 ± 0.2	100.5	1.06

[a] Regression line was $y = 118.64x + 190.61$ with a correlation coefficient of 0.9954.

Table 6
Results of analysis of commercial formulations containing ascorbic acid by the proposed and reference methods (the monographs establish that the formulations must contain not less than 90.0% and no more than 110.0% of the labeled amount of ascorbic acid).

	Measured concentration ± SD		Accuracy (% recovery)		Precision (% R.S.D.)	
	UV method	HPLC method	UV method	HPLC method	UV method	HPLC method
Redoxon® Drops (declared value: 200 mg mL^{-1})						
TEA solution	165 ± 1	162 ± 1	82.5	81.0	0.87	0.69
TEA:HP-β-CD solution	177 ± 1	178.6 ± 0.9	88.5	89.3	0.62	0.53
Reference method	177 ± 2		88.5		1.26	
Redoxon® Effervescent Tablets (declared value: 2 g)						
TEA solution	1.79 ± 0.02	1.98 ± 0.03	89.5	99.0	0.86	0.53
TEA:HP-β-CD solution	1.96 ± 0.01	1.98 ± 0.02	98.0	99.0	0.60	0.56
Reference method	1.81 ± 0.04		90.5		2.21	
C-Vitamin Effervescent Tablets (declared value: 1 g)						
TEA solution	0.94 ± 0.01	0.93 ± 0.02	94.0	93.0	0.74	0.62
TEA:HP-β-CD solution	1.01 ± 0.01	0.989 ± 0.007	101.0	98.9	0.55	0.52
Reference method	0.94 ± 0.03		94.0		3.19	
Tanvimil C Tablets (declared value: 500 mg)						
TEA solution	510 ± 4	499.9 ± 0.2	102.0	100.0	0.71	0.04
TEA:HP-β-CD solution	504 ± 3	500.9 ± 0.2	100.8	100.2	0.54	0.05
Reference method	501 ± 6		100.2		1.24	

The R.S.D. values, for the two systems, on intra-day and inter-day experiments were found to be within the acceptable limits less than 2.0%, indicating that the methods were sufficiently precise. Besides, good recoveries of ascorbic acid were obtained at each concentration (within 100 ± 2%), suggesting the accuracy of the proposed methods.

The HPLC method was applied to the analysis of solutions of ascorbic acid in water to verify differences in the results obtained with and without the addition of ligands. As shown by the data in Table 5, significant differences were obtained in presence and absence of TEA alone or in combination with HP-β-CD in the samples analyzed, which confirmed that the method has poor accuracy and precision without addition of the ligands, indicating that the drug is degraded during the time required to perform the chromatographic run using an autosampler.

3.4. Analytical applications

In order to check the applicability of the methods in the determination of ascorbic acid, these techniques were used to analyze different commercial pharmaceutical formulations. The results obtained by the proposed methods were also compared with those produced by using the reference method, at the 95% confidence level [28].

The official methods, which are volumetric titrations [24], are based on the direct titration of ascorbic acid with 2,6-dichlorophenolindophenol. However, as the titrant acted as a self-indicator, the method was not applicable to colored samples due to the experimental error in determining the end point of titration. Consequently, as a reference method, the determination of ascorbic acid was carried out with iodine, using starch as the indicator [24].

As shown in Table 6, the analytical results of ascorbic acid in the commercial formulations analyzed by UV spectrophotometric and chromatographic methods were found in the range of 82.5–102.0% and 81.0–100.2%, respectively, compared to the declared values. The recovery measurements showed that the mean percentages of ascorbic acid in the drops were lower than those of the allowed range, suggesting degradation of the vitamin in this formulation. Nevertheless, for the other formulations, the recovery percentages determined were within the established limits of variability. In terms of precision, however, the reported R.S.D. values reflected the high precision of the proposed methods when they are applied to the assay of commercial pharmaceuticals, in comparison with those obtained by the reference method. We can notice slightly better values for HPLC in comparison with those obtained by UV.

In the particular case of effervescent tablets, the recoveries obtained in the presence of TEA alone and in the mixture of TEA:HP-β-CD suggest that the use of the combination of ligands provides a selective and specific method for the analysis of this kind of pharmaceutical dosage form, in contrast to the TEA solution. For the tablets, recovery was found to be close to 100%. The calculated *t*- and *F*-values demonstrated that the data obtained by applying the systems proposed here were statistically comparable to those provided by the reference method. Therefore, these results show that the spectrophotometric and chromatographic methods are highly accurate and precise and constitute a valid tool for the determination of AAS in tablets.

Furthermore, these studies confirm that the formation of complexes allows reliable analytical results to be obtained, due to the increase in the stability and the photostability of ascorbic acid during the preparation of the samples and their later analytical assay.

Even though the described methods, using the formation of complexes and UV spectrophotometry or HPLC, showed excellent precision and accuracy, better results were achieved by using the HPLC method. Moreover, these techniques can be successfully applied for the routine analysis and quality control of pharmaceutical preparations containing this drug. This represents a great advance on the titration method, which requires more preparation time and standardization of the titration solutions in order to determine the exact final point.

4. Conclusion

The formation of binary and multicomponent complexes strongly reduced the photodegradation process of ascorbic acid. All the systems studied showed a high degree of protection from light, with photodegradation rates always lower than that of the free molecule in solution under the same experimental conditions. Furthermore, the results indicated a synergistic effect between HP-β-CD and TEA on the photodegradation of ascorbic acid, with these methods appearing to be of great interest for the assay of ascorbic acid, due to the fact that the solutions may be exposed to both indirect sunlight and room light over prolonged periods of time.

Spectrophotometric and chromatographic methods were developed in the presence of both ligands without loss of ascorbic acid during preparation of the analytical samples and the assay. All the validation parameters were found to be satisfactory, after which, the methods were applied to the determination of ascorbic acid in pharmaceutical formulations. The sample recoveries were in good agreement with their respective label values for the formulations. This implies that the methods were valid without any interference from the formulation excipients present in the analyzed formulations, for the assays.

Though UV spectrophotometric methods have the advantages of low cost and speed, the chromatographic ones were found to be more sensitive, accurate and precise. Moreover, the short analytical run time and therefore the lower solvent consumption leads to a cost-effective and environmentally friendly chromatographic procedure. Also, the proposed methodology is rapid and requires a simple sample preparation procedure.

In addition, the proposed methods decreased the degree of uncertainty and the time of the assay, compared to the reference one. So, they provide high throughput solution for the determination of ascorbic acid, either in the pure form or in pharmaceutical formulations.

Acknowledgements

Financial support from the FONCyT Préstamo BID 1728/OC-AR PICT 1376, the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT-UNC), are greatly acknowledged. We also thank Ferromet S.A. (agent of Roquette in Argentina) for their donation of hydroxypropyl-β-cyclodextrin. We would also like to specially thank Dr. Paul Hobson, native English speaker, for revision of the manuscript.

References

[1] H.H. Tonnesen, Int. J. Pharm. 225 (2001) 1–14.
[2] H.H. Tonnesen, M. Masson, T. Loftsson, Int. J. Pharm. 244 (2002) 127–135.
[3] G. Ragno, E. Cione, A. Garofalo, G. Genchi, G. Ioele, A. Risoli, A. Spagnoletta, Int. J. Pharm. 265 (2003) 125–132.
[4] R. Grillo, N. de Melo, C. Morales Moraes, R. de Lima, C. Menezes, E. Igne Ferreira, A. Henrique Rosa, L. Fernandes, Fraceto, J. Pharm. Biomed. Anal. 47 (2008) 295–302.
[5] G. Wenz, Chem. Int. Ed. Engl. 33 (1994) 803–822.
[6] J. Szejtli, Chem. Rev. 98 (1998) 1743–1754.
[7] A. Harada, Coord. Chem. Rev. 148 (1996) 115–133.
[8] T. Loftsson, Drug Stab. 1 (1995) 22–33.
[9] G. Granero, C. Garnero, M. Longhi, Eur. J. Pharm. Sci. 20 (2003) 285–293.
[10] G. Granero, M. Maitre, C. Garnero, M. Longhi, Eur. J. Med. Chem. 43 (2008) 464–470.
[11] L. Muller, J. Pharm. Biomed. Anal. 25 (2001) 985–994.
[12] K. Connors, A. Amidon, V. Stella, Chemical Stability of Pharmaceuticals, A Handbook for Pharmacists, 2nd ed., John Wiley & Sons, New York, 1986.
[13] C. Garnero, M. Longhi, J. Pharm. Biomed. Anal. 45 (2007) 536–545.
[14] A. Almeida, J. Pharm. Biomed. Anal. 21 (1999) 399–406.
[15] J. Lykkesfeldt, Anal. Biochem. 282 (2000) 89–93.
[16] M. Antonelli, G. D'Ascenzo, A. Lagan, P. Pusceddu, Talanta 58 (2002) 961–967.
[17] X. Wu, Y. Diao, C. Sun, J. Yang, Y. Wang, S. Sun, Talanta 59 (2003) 95–99.
[18] M. Pournaghi-Azar, R. Ojani, Talanta 44 (1997) 297–303.
[19] E. Akyilmaz, E. Dinchaya, Talanta 50 (1999) 87–93.
[20] E. Backheet, K. Emara, H. Askal, G. Saleh, Analyst 116 (1991) 861–865.
[21] J. Kwakye, Talanta 51 (2000) 197–200.
[22] K. Guclu, K. Sozgen, E. Tutem, M. Ozyurek, R. Apak, Talanta 65 (2005) 1226–1232.
[23] International Conference on Harmonization (ICH) of Technical Requirements for registration of Pharmaceuticals for Human Use, Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva, 2005.
[24] United States Pharmacopeia XXX (NF 25), United States Pharmacipeial Convention, Rockville, MD, USA, 2007.
[25] International Conference on Harmonization (ICH) of Technical Requirements for registration of Pharmaceuticals for Human Use, Topic Q1B: Stability Testing: Photostability Testing of New Drug Substances and Products, Geneva, 1996.
[26] I. Ahmad, Q. Fasihullah, A. Noor, I. Ansari, Q. Manzar, Ali, Int. J. Pharm. 280 (2004) 199–208.
[27] E. Touitou, D. Gilhar, F. Alhaique, A. Memoli, F. Riccieri, E. Santucci, Int. J. Pharm. 78 (1991) 85–87.
[28] J. Miller, J. Miller, Statistics and Chemometrics for Analytical Chemistry, 4th ed., Pearson, Prentice Hall, Harlow, England, 2000.