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A rapid and selective solid-phase UV spectrophotometric method for determination of ascorbic acid in pharmaceutical preparations and urine

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Abstract

Ascorbic acid was determined by a solid-phase UV spectrophotometry technique through the sorption of this on a dextran-type anion-exchange resin, Sephadex QAE A-25 and posterior direct measurement of its absorbance on the resin at 267 and 400 nm, packed in a 1-mm cell. The calibration graph was linear over the range 0.3–5.0 μg ml^{-1}. The sensitivity obtained is more than 50 times higher than that of the corresponding solution method. The detection limit was 0.05 μg ml^{-1} and the relative standard deviation 0.74% ($n = 10$). This method is very rapid and highly selective for determining ascorbic acid in the presence of other species absorbing in the ultraviolet region and which are normally encountered with it. The one-step method proposed was successfully applied in the determination of ascorbic acid in pharmaceutical preparations and urine and the results were of comparable accuracy as indicated by a statistical analysis of the data, using both *t*- and *F*-tests.

Keywords: Ascorbic acid; Solid-phase ultraviolet spectrophotometry; Pharmaceuticals preparations; Urine

1. Introduction

The determination of ascorbic acid in various matrices has been carried out by several analytical techniques, HPLC [1–5] and spectrophotometry being the most common techniques applied.

Spectrophotometric determination of ascorbic acid by direct measurement of its absorption in the ultraviolet region is very difficult as ascorbic acid is usually found in solutions containing substances also absorbing in this region. The problem with interferences in the UV region from these substances has been overcome by the use of reagents which have specific colour reactions with L-ascorbic acid. Most of these methods are based on the reducing ability of ascorbic acid [6–9] and consequently are subject to limitations as other reducing substances can interfere with the determination, and tedious pretreatment is often necessary to remove possible interference.

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In this paper, the solid-phase UV spectrophotometry technique has been used for the determination of ascorbic acid by direct measurement of its intrinsic ultraviolet absorption after sorption on a solid support. The main advantages of this technique include high sensitivity, which is due to the preconcentration of the analyte on the solid support and an increase in specificity avoiding a lot of interferences because of the need for retention on the solid support. There are a lot of applications of solid-phase spectrophotometry (SPS) in the determination of different species based on the formation of coloured derivative products [10–12]. However, the applications for the determination of species by direct measurement of its intrinsic ultraviolet absorption are very rare [13–15]. Along with the advantages indicated above, this method offers an important simplicity and rapidity as it is a one-step method and it is not necessary to obtain a derivative product or a previous treatment of the sample.

The method has been applied satisfactorily to the determination of ascorbic acid in samples of different nature like pharmaceutical preparations and urine.

2. Experimental

2.1. Apparatus

A single-beam GBC 911A microprocessor-controlled UV-VIS spectrophotometer supplied by GBC Scientific Equipment Pty Ltd. with 1-mm optical path length quartz cells from STARNA was used for all absorbance measurements. This instrument was controlled via a BRAVO AST/30286 microcomputer connected by means of a serial port for data acquisition and data processing using SCAN MASTER V 1.62 program (from GBC). All of the spectra were registered with a scan rate of 250 nm min^{-1}. A COMX PL80 plotter was used for graphical representations. An Agitaser 2000 rotating agitator and a Selecta Model Ultrasons ultrasonic bath were also used for stirring the glass tubes and dissolving the samples, respectively.

2.2. Reagents

All chemicals used were of analytical-reagent grade and doubly distilled water was always used.

2.2.1. L(+)-ascorbic acid solutions (Panreac)

Only aqueous fresh solutions, prepared from a 100 mg l^{-1} solution (daily prepared), were used for the spectrophotometric measurements. The 100 mg l^{-1} stock solution was stable for at least 6 h stored at 4°C.

2.2.2. pH Buffer solution.

A total of 0.952 g of $Na_2B_4O_7$ $10H_2O$ were dissolved in 80 ml of doubly distilled water, the pH was adjusted to $\sim$8.0 by adding 4 M hydrochloric acid, and the solution was diluted to 100 ml.

2.2.3. Sephadex QAE A-25 (Aldrich) ion-exchanger (40–120 μm)

The resin was used in the chloride form in its original dry state in order to avoid any contamination.

2.3. Absorbance measurements

After ascorbic acid was sorbed on Sephadex QAE A-25 resin, it was introduced into a 1 mm optical quartz cell and the absorbance was measured directly on it at two wavelengths, 267 and 400 nm, A_{267} and A_{400} (the former corresponding to the absorption maximum and the latter included in the range where only the resin absorbs light) against a 1 mm cell packed with resin previously equilibrated with a blank solution. The net absorbance A, was obtained by the difference between both values: $A = A_{267} - A_{400.}$

2.4. General procedure

An adequate volume of aqueous solution containing 3.0–50.0 μg of ascorbic acid was placed in a 25 ml glass tube with stopper and 1 ml of buffer solution, pH 8.0 was added; the volume was made up to 10 ml by adding doubly distilled water, 40 mg of Sephadex QAE A-25 ion-exchange resin were added to the solution and the mixture was

stirred for 5 min. The solution was filtered and the resin beads were collected together with a little volume of solution and put into a 1 mm quartz cell with the aid of a little pipette. Finally, the absorbance was measured at 267 and 400 nm as indicated in Section 2.3. A blank solution without ascorbic acid was prepared in the same manner.

A calibration graph was constructed in the same way using ascorbic acid solutions of known concentration.

2.5. Procedure for pharmaceutical preparations

An accurately weighed amount of powder obtained from the effervescent tablets or the granular packets was dissolved in water and sonicated for 5 min. The solution was filtered through a 0.45 μm membrane filter (Millipore) in instances where insoluble matter remained. The solution was then transferred into a 500 ml calibrated flask and the volume was made up to the mark with doubly distilled water.

2.6. Procedure for urine

Urine from various people was received 2 h after having an appropriate amount of the pharmaceutical preparation Citrovit, containing 1000 mg of ascorbic acid, dissolved in 50 ml of water. An equivalent volume of 0.6 M trichloroacetic acid solution was added in order to deproteinize the urine [16] and then the sample was filtered through a 0.45 μm membrane filter (Millipore). The analysis was performed immediately after diluting quantitatively to an appropriate volume.

3. Results and discussion

3.1. Spectral characteristics

In order to choose the most convenient solid support, several dextran-type resins were tested, Sephadex SPC-25, CMC-25, G-100, DEAE A-25, QAE A-25, and G-25. Dowex resins were not tested due to their high background which does not allow to work in ultraviolet region. Sephadex QAE A-25 was chosen as ascorbic acid was quickly and quantitatively sorbed on it and the absorbance obtained was the highest. Whilst ascorbic acid has an absorption maximum at 262 nm in solution, when it is sorbed on the resin, a weak bathochromic effect is produced and this maximum is displaced up to 267 nm. This fact is usual in SPS and it can be explained by the change in the environment surrounding the analyte in the solid phase with respect to the solution [15]. Due to the preconcentration of ascorbic acid on the solid support, a great increase in sensitivity is obtained.

3.2. Optimization of experimental variables

A series of experiments were conducted to establish optimum analytical variables. The parameters optimized included pH, nature of buffer solution, stirring time and amount of resin.

The effect of pH was investigated over the range 2.0–10.0. It was found that the absorbance obtained is independent of pH from 7.0 to 9.0. At pH values below and above these, a significant decrease of the absorbance is observed. Four different buffer solutions (KH_2PO_4/NaOH, NH_3/NH_4Cl, $Na_4P_2O_7$/HCl and $Na_2B_4O_7$/HCl) were tested and the latter was found to be the most appropriate (pH = 8.0). A constant absorbance was observed in the buffer concentration range studied (9×10^{-4}–4.5×10^{-3} mol l^{-1}), so that a concentration of 3×10^{-3} M of the buffer solution was selected to obtain an adequate buffering capacity.

The absorbance is practically independent of the stirring time from a value of 3 min, a maximum absorbance is reached and this one maintains constant up to 20 min (the highest stirring time value assayed). Consequently, it can be said that the fixation of the analyte on the resin is very quick. The stirring time chosen for posterior experiences was 5 min. The species fixed on the resin is stable for 20 min after equilibration.

The amount of resin employed to sorb the analyte is determined in SPS. The smaller the amount of resin used, the higher increase in sensitivity obtained, as the concentration of the analyte on the solid support is higher (Fig. 1(a)). The limit of resin which can be used is the one neces-

sary to fill up the cell up to a sufficient height, thus permitting the light beam to pass through the resin layer (40 mg in this case). When absorbance A is represented versus $1/m_r$, it is found that absorbance decreases according to the empirical equation $A = 0.055 + 0.023/m_r$ (Fig. 1 (b)).

3.3. Analytical features

According to the proposed procedure, the calibration graph was established with standard solutions of ascorbic acid and it was found that the absorbance was proportional to the amount of ascorbic acid in the range 0.3–5.0 μg ml^{-1}, with a small intercept; the regression equation obtained was $A = 0.023 + 0.187c$ (μg ml^{-1}), $r = 0.9985$. The apparent molar absorptivity was 3.29×10^5 l mol^{-1} cm^{-1}. Ascorbic acid was also determined by direct measurement of its ultraviolet absorption in solution ($\lambda = 262$ nm) and a lineal range 5–30 μg ml^{-1} was found; the regression equation was $A = 0.012 + 0.034c$ (μg ml^{-1}), $r = 0.9998$, the molar absorptivity being 6.0×10^3 l mol^{-1} cm^{-1} (Fig. 2). A high increasing in sensitivity when solid phase UV spectrophotometry is used is evident.

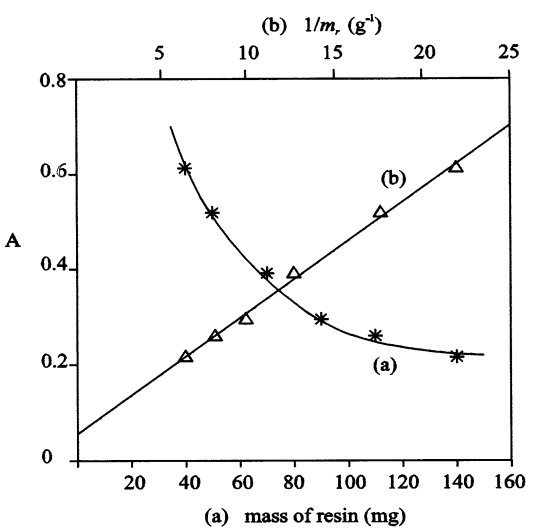

Fig. 1. Absorbance vs. (a) amount of resin; (b) inverse of amount of resin. [Ascorbic acid] = 1.70×10^{-5} M, $t_{agit.} = 5$ min.

The reproducibility of the proposed method was checked by ten independent replicate analyses on a solution containing 3 μg ml^{-1} of ascorbic acid; the relative standard deviation was 0.74% and the relative error ($P = 0.05$) of the method was 1.14%. The IUPAC detection limit ($k = 3$) [17] and the quantification limit ($k = 10$) [18] were 0.053 and 0.176 μg ml^{-1}, respectively.

3.4. Interferences

The effect of other vitamins, foreign ions and common tablet fillers in the determination of 30 μg of ascorbic acid was studied to determine the tolerance of the method to these foreign species (amount of foreign species that produces an error not exceeding ± 5%). If interference occurred, the ratio foreign species/ascorbic acid was progressively reduced until interference ceased. The proposed method is not based on the reducing ability of ascorbic acid like most conventional methods, it is less subject to reducing agent interferences. No interference was found with the presence of paracetamol, glucose, saccharose, saccharine, codeine and other tablet excipients at ratios [interferent]/[ascorbic] (w/w) much higher than those found commonly in pharmaceuticals (Table 1). When tolerance levels in homogeneous solution and in SPS methodology are compared, it is

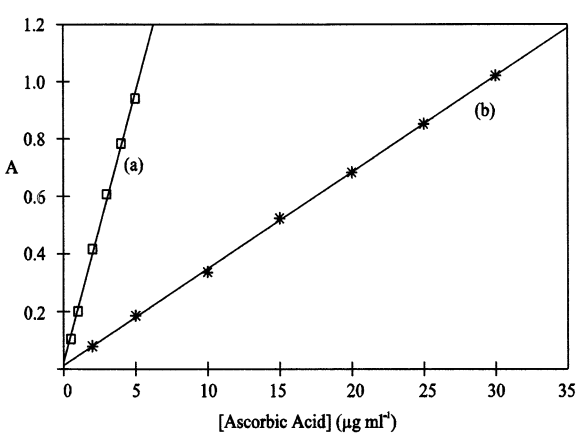

Fig. 2. Standard calibration graphs. (a) SPS method. 1-mm optical path length cell, 40 mg of resin, $\lambda = 267$ nm, pH 8.0; (b) in homogeneous solution. 1-cm optical path length cell, $\lambda = 262$ nm, pH 8.0.

Table 1
Tolerance to foreign species in the determination of ascorbic acid[a]

Species added	Tolerable [interferent]/[analyte] ratio (w/w)
Glucose, saccharose	>325[b]
Cl^-, Ca(II), Na(I), Mg(II), K(I)	>200[b]
Glutamic acid	40
Vitamin B_{12}, Vitamin B_1, Vitamin B_6	>20[b]
Codeine, caffeine	20
Citric acid	10
Paracetamol	5
Uric acid, urea	2
Acetylsalicylic acid, saccharine	0.2

[a] Ascorbic acid concentration 3.0 μg ml^{-1}.
[b] Maximum ratio tested.

found that the last ones are much higher (Fig. 3). Therefore, the solid support acts increasing the selectivity as only those species that can be sorbed on it in working conditions could interfere.

3.5. Application to real samples

In order to check the applicability of the proposed method in the determination of ascorbic acid in various samples, this was used to analyse several commercial pharmaceuticals preparations of the Spanish Pharmacopeia in different physical presentations and urine. In every case, the official method of AOAC [16] was used as a reference method; this method is a titrimetric procedure based on the reduction of the indicator dye 2,6-dichloroindophenol by ascorbic acid. The results obtained are included in Tables 2 and 3. The Student t-test and the variance-ratio F-test [19] show that there is no significant difference between the two methods with regard to accuracy and precision. As can be seen, the results of the proposed method are in accordance with those of the reference. In the case of pharmaceutical preparations, all values are in good agreement with those supplied by the manufacturers. In all instances, the samples were analysed with no pretreatment.

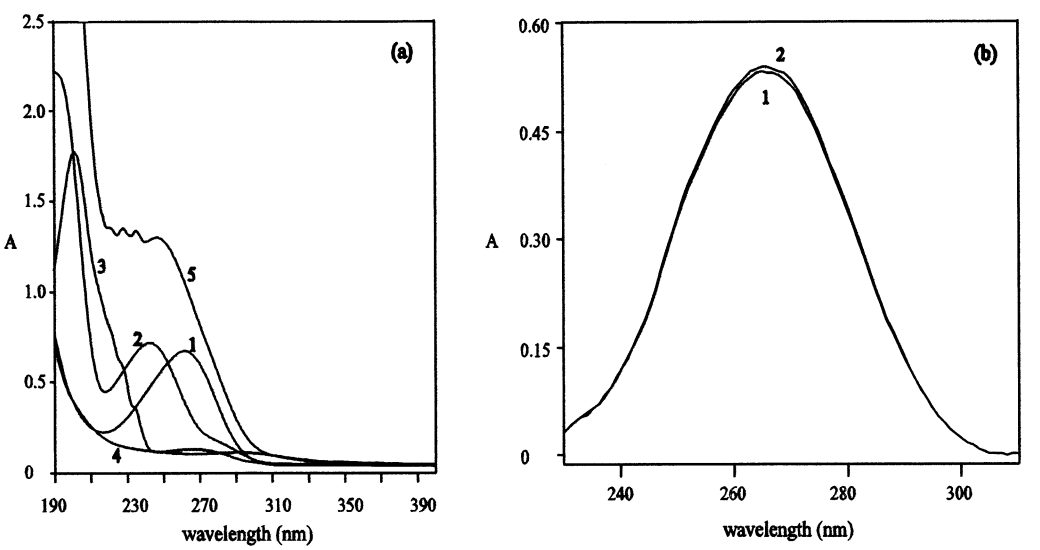

Fig. 3. Effect of other species accompanying ascorbic acid in its absorption spectrum: (a) in solution (pH 7.5, 1-cm optical path length cell): (1) 10 μg ml^{-1} ascorbic acid, (2) 10 μg ml^{-1} paracetamol, (3) 10 μg ml^{-1} saccharine, (4) 10 μg ml^{-1} acetylsalicylic acid, (5) 10 μg ml^{-1} each of ascorbic acid, paracetamol, saccharine and acetylsalicylic acid; (b) in resin (pH 7.5, 1-mm optical path length cell, 40 mg of resin): (1) 2.5 μg ml^{-1} ascorbic acid, (2) 2.5 μg ml^{-1} each of ascorbic acid, paracetamol, saccharine and acetylsalicylic acid.

Table 2
Determination of ascorbic acid in urine

Sample	Ascorbic acid amount found/mg per 100 ml		t_{calc}[a]	F_{calc}[b]
	Proposed method[c]	Reference method		
Urine 1	108.0 ± 2.4	109.0 ± 2.9	1.14	1.46
Urine 2	98.8 ± 1.9	99.2 ± 2.5	0.55	1.73
Urine 3	102.1 ± 2.0	101.8 ± 2.2	0.43	1.21
Urine 4	88.9 ± 1.8	89.6 ± 2.0	1.12	1.24

[a] Theoretical value for $t = 2.77$ ($P = 0.05$).
[b] Theoretical value for $F = 32.00$ ($P = 0.05$).
[c] Standard addition calibration graph method; average of three determinations.

Table 3
Determination of ascorbic acid in pharmaceutical preparations

Pharmaceutical	Ascorbic acid stated (g)	Ascorbic acid found/g[a]		t_{calc}[b]	F_{calc}[c]
		Proposed method	Reference method		
Redoxón[d]	1	1.002 (± 0.008)	0.993 (± 0.004)	1.65	3.35
Citrovit[e]	1	1.009 (± 0.006)	1.009 (± 0.003)	0.25	2.92
Cebión 500[f]	0.500	0.502 (± 0.007)	0.495 (± 0.002)	1.44	16.22
Algidol[g]	0.500	0.502 (± 0.005)	0.498 (± 0.004)	1.07	2.48
Lema ERN C[h]	4.5	4.52 (± 0.05)	4.54 (± 0.04)	0.54	1.56
Calmanticold Vit C[i]	0.250	0.248 (± 0.003)	0.252 (± 0.002)	1.92	2.25
Efferalgan Vit C[j]	0.200	0.202 (± 0.004)	0.198 (± 0.005)	1.07	2.48

[a] Theoretical value for $t = 2.77$ ($P = 0.05$).
[b] Theoretical value for $F = 32.00$ ($P = 0.05$).
[c] Mean ± standard deviation for three replicates.
[d] Each tablet of Redoxon (from ROCHE NICHOLAS, Barcelona, Spain) contains 1000 mg vitamin C, 20 mg sodium saccharine and 1305 mg saccharose.
[e] Each sachet of Citrovit (from ABELLÓ, Madrid, Spain) contains 1000 mg vitamin C, 7.09 g saccharose, 0.599 g glucose, 0.178 g invert sugar, 10 g excip. q.s.
[f] Each granular packet of Cebión 500 (from E. MERCK-Darmstadt IGODA, Barcelona, Spain) contains 500 mg vitamin C, 4.2 g saccharose, 6 g excip. q.s.
[g] Each granular packet of Algidol (from BERENGUER-INFALE, Prodesfarma Group Laboratories, Barcelona, Spain) contains 650 mg paracetamol, 10 mg codeine phosphate, 500 mg vitamin C, 5.5 g excip. q.s.
[h] Each syrup flask of Lema Ern C (from ERN, Barcelona, Spain) contains 4.5 g vitamin C, 65.6 g sodium borate monohydrate, 200 mg saccharine.
[i] Each granular packet of Calmanticold Vit C (from CALMANTE VITAMINADO, Córdoba, Spain) contains 250 mg vitamin C, 500 mg paracetamol.
[j] Each tablet of Efferalgan Vit C (from UPSA MÉDICA, San Sebastián, Spain) contains 200 mg vitamin C, 330 mg paracetamol.

3.5.1. Urine

Table 2 gives the ascorbic acid content in various urine samples analysed. As the urine samples taken were found to be free from ascorbic acid, these were prepared by means of ingestion of a pharmaceutical preparation containing 1000 mg of ascorbic acid (see Section 2.6). Due to the high sensitivity of the method proposed and the consequent possibility of diluting largely the sample, no interference was found despite the presence of a very complex matrix of the samples as indicated by the good agreement of the results obtained from both the proposed and the reference method.

Table 4
Comparison of sensitivities and linear dynamic ranges of spectrophotometric methods for ascorbic acid determination

Reagent	ε (l mol^{-1} cm^{-1})	Linear range (μg ml^{-1})	References
2,6 dichloroindophenol	—	1–20	[20]
p-aminobenzoic acid	—	20–67	[21]
Fast red AL salt	4.07×10^3	5–25	[22]
Iodate-fluorescein	8.81×10^3	<4.0	[23]
Fe(III)-bipyridyl	1.73×10^4	—	[24]
Fe(III)-1,10-phenanthroline	2.20×10^4	—	[7]
Fe(III)-TPTZ[a]	4.43×10^4	—	[25]
Fe(III)-ferrozine	5.58×10^4	0.2–10	[8,9]
UV spectrophotometry (conventional)	6.0×10^3	2.5–30	This paper
Solid-phase UV spectrophotometry	3.29×10^{5b}	0.3–5.0	This paper

[a] TPTZ: 2,4,6-tripyridyl-s-triazine.
[b] Apparent molar absorptivity.

3.5.2. Pharmaceutical preparations

The method proposed was applied to the analysis of several dosage forms containing ascorbic acid either in tablet or granular packets without interference from other drugs and excipients encountered. The results are summarized in Table 3. All of them agree with the reported values and those obtained by the reference method.

4. Conclusions

The one-step solid-phase UV spectrophotometric method proposed allows a very simple, rapid and direct determination of ascorbic acid in the presence of a lot of other species absorbing in ultraviolet region, and there is no need to do a previous treatment of the sample. The use of a convenient solid support strongly increases the selectivity of the procedure to most of those species usually found along with ascorbic acid with respect to its determination by conventional UV spectrophotometry in homogeneous solution. This method was compared with other spectrophotometric procedures (selected among the most sensitive reported so far) and was found to be faster and of a comparable or better sensitivity (Table 4). If the proposed method is compared with most of the last reported HPLC methods [1–4], that results to be more sensitive and precise than these ones; it is also necessary to take into account the fact that the instrumentation used is much cheaper. The method can be readily adapted to routine analyses of ascorbic acid as each determination takes only a few minutes.

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