

High Performance Liquid Chromatographic Method for Determination of Ascorbic Acid in Pure Form, Pharmaceutical Formulations, Biological Samples and in Environmental Water Samples

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Abstract:

A simple, precise, rapid, and accurate reversed-phase high performance liquid chromatographic method has been developed for the determination of ascorbic acid in pure form, pharmaceutical formulations, biological samples and in environmental water samples.

Chromatography was carried out on supercar L₁ (C₁₈) reversed- phase column (25cm×4.6mm), 5 microns, using a mixture of methanol : water (42: 58v/v) as a mobile phase at a flow rate of 1.0 ml.min⁻¹. Detection was performed at 254nm at ambient temperature.

The retention time for ascorbic acid was found 2.3 minutes. The calibration curve was linear (r= 0.9998) over a concentration range from 0.5 to 12.5µg/ml. Limit of detection (LOD) and limit of quantitation (LOQ) were found 3ng/ml and 9 ng/ml respectively. The method was validated for its linearity, precision and accuracy.

The proposed method was successfully applied for the determination of ascorbic acid in pure form, pharmaceutical formulations, and biological samples and in environmental water samples.

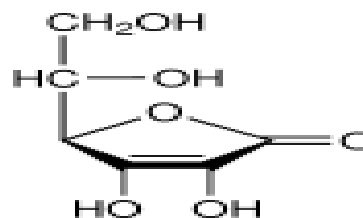
Keyword: HPLC, Ascorbic acid, Pharmaceutical formulations.

الخلاصة:

تم اختبار طريقة كروماتوغرافيا السائل ذات الأداء العالي لتقدير فيتامين سي بحالته النقية و بعض مستحضراته الصيدلانية والنماذج البيولوجية وفي نماذج من المياه. تميزت الطريقة بالبساطة والدقة والسرعة والضبط العالي, حيث تم الفصل باستخدام عمود نوع L1 و استخدام مزيج الميثانول - الماء كوسط ناقل بنسبة (42:58) حجم/حجم وبسرعة جريان 1 مل/دقيقة واستخدام مكشاف الأشعة فوق البنفسجية عند الطول الموجي 254 نانومتر وفي درجة حرارة المحيط , وأمكن تقدير الكميات التي تتراوح بين 0.5-12.5 مايكروغرام/مل وبحددي كشف وكمي هما 3 و9 نانوغرام/مل على التوالي واختبر مصداقية الطريقة بقياس استقامة الخط البياني والضبط والدقة واستخدمت الطريقة بنجاح لتقدير حامض الاسكوربيك في حالته النقية. مستحضراته الصيدلانية, النماذج البيولوجية وفي نماذج من المياه.

Introduction:

Ascorbic acid, the enolic form of 3-oxo-L-gulofuranolactone ^[1] (figure-1), a water-soluble more commonly known as vitamin C which is an important vitamin having a chemical structure that justifies its classification as a carbohydrate, and is important in forming collagen, a protein that gives structure to bones, muscles and blood vessels.



L-threo-Hex-2-enono-1,4-lactone
Molecular formula: C₆H₈O₆ =176.1

Figure-1: Chemical structure of ascorbic acid.

The deficiency of this vitamin leads to many diseases: scurvy, plug poisoning, liver disease, allergic reactions, arteriosclerosis, etc. So that is widely used in the treatment of certain diseases such as scurvy, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility^[1-4].

It is one of the most ubiquitous vitamins ever discovered. In addition, ascorbic acid has been widely used in the pharmaceutical, chemical, cosmetic and food industry as antioxidant. Therefore, there is a need to find an accurate, reliable, rapid, and easy method for measuring the amount of ascorbic acid in a sample. However, various methods have been employed for the quantitative determination of ascorbic acid in pharmaceutical formulations, fruit juices, urine, plasma etc.

These include titration^[5-10], fluorimetry^[11], spectrophotometry^[12-17], differential pulse voltammetry^[18-19], square-wave voltammetry^[20-21], cyclic voltammetry^[22], capillary zone electrophoresis^[23], chemiluminescence^[24] and high-

performance liquid chromatography (HPLC)^[25-28].

High performance liquid chromatography (HPLC) can be used for determination of drugs and for purposes of control throughout the entire manufacturing process of drugs as well as quality control of the finished product.

It has the advantages of being sensitive, selective, rapid, accurate and reproducible.

The main objective of the present study was to optimize a simple and rapid HPLC method for the determination of ascorbic acid in pharmaceutical preparations, biological samples and in environmental water samples.

Materials and Methods:

Experimental Apparatus:

Chromatographic system consisted of a shimadzu HPLC model LC-20AT with UV detector model SPD-20A and L₁ supelco column (25cm×4.6mm), 5 μm particle size. HPLC conditions are given in table-1.

Table-1: HPLC conditions

Column	Supelco L ₁ (25cm×4.6mm),5 μm
Wavelength	254nm
Mobile phase	Methanol- H ₂ O
Retention time	2.3min
Flow rate	1.0ml/min
Temperature	Ambient
Injection volume	10 μL

Reagents:-

All chemical used were of analytical or pharmaceutical grade and HPLC grade methanol from Merck (Darmstadt, Germany) was used throughout.

Ascorbic acid standard material was provided from the state company for pharmaceutical industries (HPI) Mosul-Iraq.

Preparation of standard solutions:

A standard stock solution of ascorbic acid (100μg/ml) was prepared in

mobile phase. Working standard solutions in a range of (0.5-12.5μg/ml) were prepared by dilution from stock solution.

Preparation of mobile phase:-

The mobile phase was prepared by mixing methanol, water, in the ratio of 42: 58v/v.

HPLC method for determining ascorbic acid:

A series of standard solution containing 0.5-12.5μg/ml of ascorbic acid and the sample solution of pharmaceutical preparation were applied respectively. 10μl

an aliquot of each solution was injected on to the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of ascorbic acid.

The concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from the concentration and peak area data.

Procedures for pharmaceutical preparations (syrups):

Two different marketed ascorbic acid syrup formulations (Coldin 50mg/5ml, Pulmocodin 12.5mg/5ml, were selected for analysis).

The content of 5 bottles was mixed well in 1L dried beaker.

Aliquots equivalent to 10 mg of ascorbic acid were transferred into 1L volumetric flasks and diluted with mobile phase to the volume. And the amount of ascorbic acid was determined by comparing the peak area of the assay preparation with the standard preparation at the same concentration.

Procedure for pharmaceutical preparations (tablets):

Two different marketed ascorbic acid tablets formulations (Flu-out 100mg, ascorbic acid tablet 250mg), were selected for analysis. To minimize a possible variation in the composition of the tablet, the mixed content of 20 tablets were weighed and grounded, then the powder equivalent to 5 mg of ascorbic acid in to 500 ml volumetric flask. Added about 100 ml mobile phase and mixed well for 30 minute to dissolve, completed to the volume with mobile phase. Filtered and then determination of ascorbic acid as described under recommended procedure.

Procedure for biological samples:

Since the presence of ascorbic acid has been reported in blood and urine samples^[29].

The method was applied for its determination in these samples. 5mL of blood and urine samples were taken from

pathology laboratory [Nineveh-Iraq] and 1 mL of 5% EDTA, 2 mL of 1% (tri chloro acetic acid) and 2mL of 3% meta phosphoric acid were added to the analyte, centrifuged, the supernatant was diluted to a suitable volume with mobile phase, and the amount of ascorbic acid was determined by calculated from the regression equation derived from the concentration and peak area data.

Procedure for water samples:

The tap and river water samples were found to be free from ascorbic acid, synthetic samples were prepared by adding known concentration of ascorbic acid to each sample prior analysis in the range from 1-10 ppm the determination of ascorbic acid proceeded as described under HPLC method for determining ascorbic acid. Calculate the percentage recovery using a calibration graph previously prepared.

Results and Discussion:

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and pharmaceutical products.

The aim of this study was to develop a rapid HPLC method for the determination of ascorbic acid in pure form, its pharmaceutical formulations, and biological samples and in environmental water samples, using the most commonly employed RP C₁₈ column with UV detection. The detection wavelength of 254nm was chosen in order to achieve a good sensitivity for quantitative determination of ascorbic acid in syrups, tablets, blood, urine and water.

The mobile phase consisting of methanol: water (42:58) offered a good separation at ambient temperature under these conditions using a flow rate of 1.0 ml/min and retention time of 2.3 min as shown in the chromatogram, figure-2.

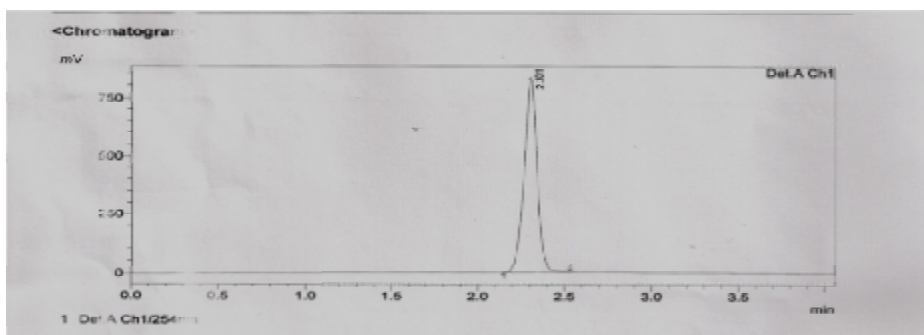


Figure-2: Typical chromatogram (ascorbic acid 10µg/ml).

Under the described experimental conditions the analyte peaks were well defined and free from tailing. Ascorbic acid was determined by measuring the peak area.

A plot of peak area against concentration gave a linear relationship

($r=0.999$) over the concentration rang 0.5-12.5µg/ml.

Using regression analysis, the linear equation $Y=50341x+75148$ was obtained where Y is the mean peak area and X is the concentration in µg/ml figure-3.

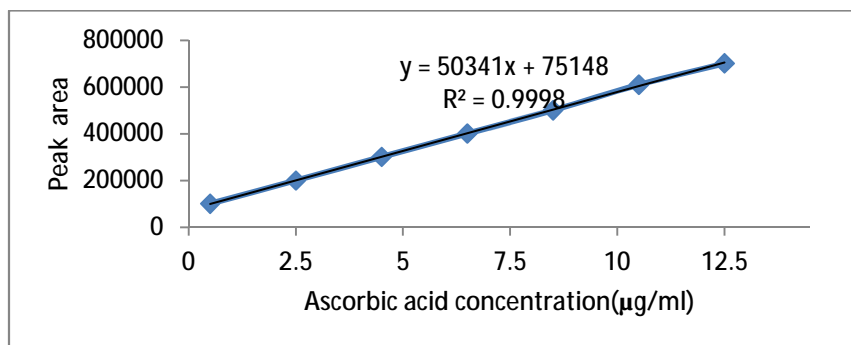


Figure-3: Calibration curve for ascorbic acid.

Determination of limit of detection and limit of quantization (sensitivity). The standard deviation at concentration 0 (only mobile phase as blank) was calculated and this value was used for the calculation of the limit of detection and limit of quantitation.

The limits of detection (LOD) and quantification (LOQ) were calculated using the following formulae: $LOD= (3.3\sigma/s)$ and $LOQ= (10 \sigma/s)$ where σ is the standard deviation of the response of blank and is the slope of the regression line ^[30].

Limit of detection (LOD) and limit of quantification (LOQ) were found 3ng/ml and 9ng/ml respectively.

Method precision:

The precision of the method was established by carrying out the analysis of ascorbic acid (n=6) using the proposed method .The low value of standard deviation showed that the method was precise. The results obtained were presented in table-2.

Table-2: Method precision.

Ascorbic acid concentration(µg/ml)	Amount found (µg/ml)	% Assay Mean (n=6)	%RSD of Assay (n=6)
1.0	1.012	101.2	0.8
6.0	6.102	101.7	1.1
10.0	9.99	99.9	0.9
Mean =	100.933		0.933

Method accuracy:

To ensure the accuracy of the method, recovery studies were carried out at three different levels.

The Results of recovery studies were found to be satisfactorily high, mean recoveries being 100.222 ± 0.278 (n=3) as shown in table-3.

Table-3: Method accuracy.

Ascorbic acid /Amount(μg)	Amount found (μg)	%Recovery n =3
2.0	2.01	100.5
6.0	5.995	99.916
10.0	10.025	100.25
Mean=		100.222 ± 0.278

Analytical application:

The proposed method was successfully applied to the assay of ascorbic acid in syrups, tablets, and blood, urine and water samples.

The result of analysis for pharmaceutical formulations shown in

table-4, which reveals that there is close agreement between the results obtained by the proposed method and label claim, the results of biological samples (blood and urine table-5) and the results of water samples (table-6) show that the recovery values obtained were closed to 100%.

Table-4: Determination of ascorbic acid in Pharmaceutical formulations.

Pharmaceutical Formulations (HPI)	Label amount (mg)	Found* (mg)	% Recovery
Flu-out tablet	100 mg/tab	100.3	100.3
Ascorbic acid tablet	250 mg/tab	249.85	99.94
Coldin syrup	50mg/5ml	49.96	99.92
Pulmocodin syrup	12.5mg/5ml	12.54	100.32

* Mean value of six determinations.

Table-5: Determination of ascorbic acid in blood and urine samples.

Sample	Ascorbic acid originally found ($\mu\text{g/ml}$) (A)	Ascorbic acid added($\mu\text{g/ml}$) (B)	Total ascorbic acid found($\mu\text{g/ml}$) (C)	Difference (C-A)	Recovery% [◀] (C-A/B)X100
Blood	0.8	10	10.84	10.04	100.4
Urine	0.7	10	10.72	10.02	100.2

* Mean value of five determinations

Table-6: Determination of ascorbic acid in water samples.

Water samples	Ascorbic acid(mg/ml)		% Recovery(n=10)
	taken	found	
Tap water	1.0	0.994	99.4
	3.0	3.002	100.066
	10.0	0.9992	99.92
River water	1.0	0.996	99.66
	3.0	0.2995	99.83
	10.0	10.003	100.03

Conclusion:

In this study, a simple, rapid, sensitive, accurate, precise and economical HPLC method was developed and validated for the determination of ascorbic

acid in syrups, tablets, blood, urine and water samples.

The method was selective using C₁₈ (L1) analytical column and applicable to different samples.

Thus the developed method is recommended for control throughout the entire manufacturing process of drugs as well as quality control of the finished product in industry, research laboratories and hospitals, in view of its high recovery and precision.

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