REVIEW ARTICAL

DEVELOPMENTS IN THE CLINICAL AND FOOD ANALYSIS OF VITAMIN C

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ABSTRACT:

Vitamin C (ascorbic acid) is a water-soluble vitamin that is necessary for the prevention and cure of the deficiency disease scurvy. It plays an important role as redox cofactor and catalyst in a broad array of biochemical reactions and processes. The redox behavior of ascorbic acid is the basis of most of the analytical methods used for its determination. The various methods developed for the determination of ascorbic acid in biological samples and food materials include spectrophotometric methods, fluorimetric methods, mass spectrometric methods, chromatographic methods and enzymatic methods. The high-performance liquid chromatographic methods using different detection systems are highly specific and sensitive for the determination of ascorbic acid and related compounds such as dehydroascorbic and isoascorbic acid in biological samples.

INTRODUCTION

Ascorbic acid (vitamin C) is a water-soluble vitamin and is necessary for the prevention and cure of the deficiency disease scurvy. It plays an important role as redox cofactor and catalyst in a broad array of biochemical reactions and processes. It is chemically known as 2-oxo-L-theo-hexono-4-lactone-2,3-enediol and has a planar five-membered ring. The chemical structures of ascorbic acid and oxidation products are given in Fig.1[1]. The various methods of analysis are largely based on the redox characteristics and ionization behavior of ascorbic acid. The important physicochemical properties of ascorbic acid[1-5] are given below:

p*K*a1	4.17
p*K*a2	11.57
Redox potential	
(dehydroascorbic acid / ascorbate)	-174 mV
(ascorbate$^-$ H^+ / ascorbate$^-$)	+282 mV
Absorption maxima	
pH 2.0	245 nm [A (1 %, 1 cm) 695]
pH 6.4	265 um [A (1 %, 1 cm) 940]

These properties have been utilized in the development of analytical methods for the determination of ascorbic acid. The most important property of ascorbic acid is the reversible oxidation to semidehydro-L-ascorbic acid and oxidation further to dehydro-L-ascorbic acid[6,7]. This property imparts physiological activity to the vitamin.

Methods of Analysis

Recent accounts of the development of analytical methods for the determination of ascorbic acid in biological samples, food materials and pharmaceuticals are reported in the literature[2,8-10]. Most of these methods are based on the application of spectrophotometric, fluorimetric and chromatographic techniques to suit the requirements of a particular analysis and are summarized below:

A. Spectrophotometric Methods:

Ascorbic acid exhibits strong absorption in the ultraviolet region, which is the basis of spectrophotometric methods for the determination of the vitamins in pure solutions and in sample preparations where no interference is observed from UV absorbing impurities. The value of A (1 %, 1cm) at the analytical wavelength of 245 nm (pH 2.0) is high (695) which makes the method very sensitive for the determination of mg quantities. Treatment of the

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material to be analyzed with ascorbic acid oxidase is often used as a blank to correct for interfering substances in biological samples[11]. A spectrophotometric method for the determination of ascorbic acid in pharmaceuticals by background correction (245 nm) has been reported[12]. The direct determination of ascorbic acid in mixtures involves the use of 2,2'-dipyridyl as a colorimetric reagent. The method is based on the reduction of Fe (III) by ascorbic acid to Fe (II) which reacts with 2, 2'-dipyridyl to form a colored complex (absorption maximum 510 nm) that can be used for quantitative determination[13]. A spectrophotometric method has been developed for the determination of ascorbic acid and its oxidation product, dehydroascorbic acid in biological samples[14]. A sensitive method has been developed for the determination of ascorbic acid in fruit juice and pharmaceutical formulations using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP)[15]. A novel UV method has been developed for the analysis of ascorbic acid in various formulations[16].

B. Fluorimetric Methods:

Fluorimetry is a highly sensitive technique for the determination of fluorescent compounds or fluorescent derivatives of non-fluorescent compounds. The technique has been used for the detection of μg quantities of the vitamin. Methods based on fluorimetric[17] and chemiluminescence detection[18] provide highly sensitive methods for the determination of ascorbic acid in plant and other materials.

C. Mass Spectrometric Methods:

Conventional and isotope mass spectrometric techniques have also been used for the analysis of ascorbic acid. Isotope ratio mass spectrometry is particularly useful and sensitive when ^{13}C ascorbic acid is used as a reference or standard in the analysis of complex matrices[19].

D. Chromatographic Methods:

High-performance liquid chromatographic (HPLC) methods have extensively been employed for the determination of ascorbic acid in biological samples. These methods include ion exchange, reversed phase and ion-pairing HPLC chromatographic protocols. Spectrophotometric, mass spectrometric, fluorimetric and electrochemical detection has been used in the HPLC analysis of ascorbic acid. The electrochemical detection is used for the simultaneous determination of ascorbic acid, dehydroascorbic acid, isomers and derivatives. A number of HPLC methods have been developed for the detection and determination of ascorbic acid, oxidation products and derivatives in biological samples and plant materials[18, 20-29]. The limit of detection of ascorbic acid in plasma or urine with UV detection lies in the range of 100-120μ g[30,31]. Fluorescence detection of ascorbic acid and dehydroascorbic acid in plasma and its comparison with coulometric detection has been reported[32].

Liquid chromatography methods based on precolumn and *o*-phenylenediamine (OPD) derivatization have been used for the determination of total vitamin C and total isovitamin C in foods and dehydro forms of the vitamin. Isoascorbic acid has been used as an internal standard[33-39]. The limits of detection of ascorbic acid by HPLC using different detectors are reported in Table-1 [40-42].

E. Enzymatic Methods:

Enzymatic methods using ascorbate oxidase are specific and have the advantage of selectively measuring the biological activity of ascorbic acid in serum or plasma [11]. Ascorbate oxidase and OPD derivatization has been used to develop a rapid automated method for the routine assay of ascorbic acid in serum and plasma. The method has a sample throughput of 100/h.[43]

F. Commercial Kits:

Commercial kits (e.g. Immunodiagnostic, Germany; Biovision, USA) are also used for the determination of ascorbic acid in serum or plasma in clinical laboratories.

DISCUSSION

The literature review presented above has pointed out that a number of physical methods based on various principles and applicable to the analytical response of ascorbic acid have been developed for its assay in biological samples and food materials. The chromatographic methods particularly HPLC is most effective in achieving the separation and simultaneous determination of ascorbic acid and its various analogues. Depending on the nature and amount of the vitamin in the clinical sample and the possible interference from related compounds various detection systems (Table-1) have been utilized for the assay of ascorbic acid. The physicochemical characteristics

Table 1:

Limits of detection of ascorbic acid in biological samples by HPLC

Media	Detector	Limit of Detection	Reference
Plasma	UV	120 μ g/L	30
Plasma/Urine	UV	100/400 μ g/L	31
Plasma	Electrochemical	240 μ g/L	40
Plasma	Fluorometric	16 μ g/L	41
Serum	UV	1.3 x 10^{-8} mol/L	42

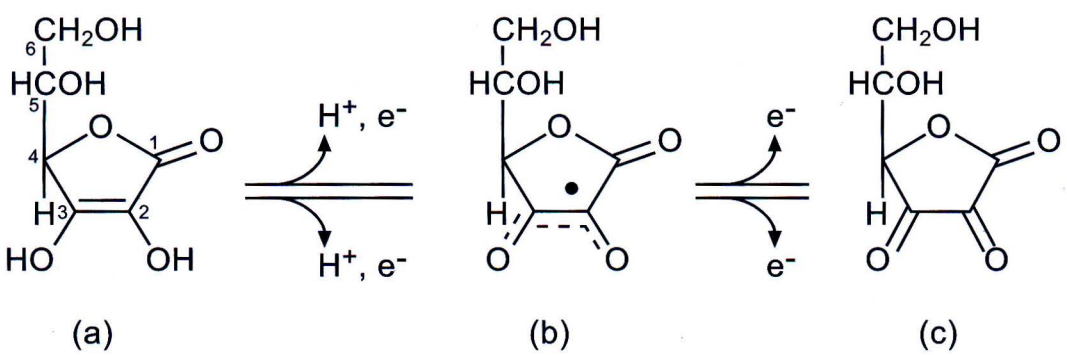

Fig. 1: Ascorbic acid and its oxidation products (a) L-ascorbic acid, (b) ascorbate radical (c) dehydroascorbic acid.

involved in these assays include the redox potential, electrooxidation and fluorescence of vitamin C.

It needs to be mentioned that the widely used spectrophotometric methods reported for the assay of ascorbic acid may not be specific and could suffer from interference by UV absorbing impurities or isomeric compounds present in the sample. It is advisable to confirm the presence of any interfering components by alternative assay methods such as HPLC to assess the accuracy of determination. In clinical samples vitamin B components with similar physicochemical characteristics might interfere unless a more specific method is available for the assay. In the case of samples containing the oxidation products of ascorbic acid (e.g. dehydroascorbic acid and gulonic acid) a stability-indicating method can only determine the ascorbic acid content accurately and should be used. The sensitivity of the assay method as determined by the detection limit (Table 1) is an important consideration in samples with low vitamin C content and the fluorescence detection may be the method of choice in such cases. With the development of instrumentation more specific and sensitive methods may be available for the assay of ascorbic acid in future.

CONCLUSION

The clinical analyst has a wide choice of methods for application to biological matrices and other systems. HPLC methods are capable of accurate differentiation between ascorbic acid and isoascorbic acid and the dehydro forms of the epimers. However, because of the variability that exists in the application of the methods between laboratories, considerable variations in assay values can occur when different laboratories assay like biological samples using proven methods of their choice[10]. The specificity and sensitivity of a method are important considerations in the determination of a drug in biological samples.

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