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Ascorbic Acid: Methods of Analysis

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ABSTRACT

Ascorbic acid (vitamin C) is an important vitamin for the maintenance of health and prevention of scurvy. Several analytical methods have been developed for the determination of ascorbic acid. These methods are based on UV and visible spectrometry, mass spectrometry, chromatography, electrochemical and titrimetric techniques. This review outlines the applications of these techniques to the assay of ascorbic acid in food materials, pharmaceutical preparations and biological fluids.

Keywords: *Ascorbic acid, Spectrometric, Potentiometry, Colorimetry, Polarimetry*

INTRODUCTION

*Vitamin C or L-ascorbic acid (AA) is a water-soluble vitamin that cures and prevents scurvy and is known for its function as a redox cofactor and catalyst in many biochemical process. There are two enantiomeric pairs of AA i.e. L- and D-ascorbic acid and L- and D-isoascorbic acid. D-ascorbic acid do not occur in nature and is devoid of vitamin C activity. L- ascorbic acid possesses biological activity and is easily and reversibly oxidized to dehydroascorbic acid (Fig. 1), which possesses vitamin C activity and is readily reduced to ascorbic acid in the animal body [1].

The physico-chemical properties of ascorbic acid are summarized in Table 1. Insufficient intake of this vitamin causes scurvy which is characterized by spongy gums, fatigue, spots on skin, bleeding from mucous membrane and connective tissues weakness [2, 3]. However the intake of citrus fruits and scurvy grass can be helpful in preventing the deficiency conditions [4]. Citrus fruits and green vegetables are the rich sources of ascorbic acid. Potatoes have higher amount and liver and kidney are good sources. Cereal grains do not contain the vitamin [5]. The levels of vitamin C in natural products are affected by the climate, sunshine, sunlight and storage.

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AA is more stable at pH values ranging from 3.0-4.5 than in the range of 5.0-7.0 [6]. The rate of oxidation is increased as the pH increases from 1.5-3.5 [7]. It is unstable at alkaline pH and is rapidly oxidized especially in the presence of trace elements. Therefore while compounding the TPN solutions it is added shortly before administration [8]. Sweetening agents enhance the photostability of ascorbic acid and hydrogen peroxide reduces its stability [9].The rate of degradation of AA increases with an increase in hydrogen peroxide concentration [10].

METHODS OF ANALYSIS

Several methods have been used for the analysis of AA. Some of the important methods based on different techniques are presented below.

Spectrometric methods

UV-Visible Spectrometry

Several UV-visible spectrometric methods have been developed for the determination of AA in food [12], pharmaceutical products [13] and

biological fluids [14, 15]. Other spectrometric methods developed for the determination of AA are as follows.

A UV spectrometric method has been developed for the determination of AA in aqueous medium by using sodium oxalate (0.0056 mol/dm^3) as stabilizer and in this method extraction procedure is not required. The molar absorptivity of AA at 266 nm is reported as 1.43×10^4 dm^3 mol^{-1} cm^{-1}. The concentration range for AA/cm^3 is 0.857-12.0 µg in which the Beer's Law is obeyed and the %RSD for the determination of 8.0 µg AA/ cm^3 (n=7) is 0.81% [16]. AA is stabilized with glycine in phosphate buffer and in aqueous solution has been determined spectrometrically. The molar absorptivity at 266 nm used in the method is 1.39×10^4 dm^3 $mol^{-1}cm^{-1}$ which is similar to that reported earlier [16]. The calibration curve is linear in the concentration range of 1.25-12.0 µg/cm^3. The limit of detection is found to be 0.375µg/cm^3 and %RSD is 0.82% for the concentration of 8.00 µg/cm^3 of L- AA [17].

An easy, fast and inexpensive method for the determination of AA in a three component drug mixture of pyridoxine HCl, tyrosine and AA has been reported. The method obeys Beer's law in the range of 7.0-15.0 µg/ml and the lower limit of detection at 95% confidence level is 2.88 µg/ml [18].

A new reaction system for the quantitative determination of AA in pharmaceutical products has been developed that is based on the inhibitory effect of AA on orange G-bromate system. The RSD for 5.0 and 20.0 µg/ml of vitamin C are 1.08 and 1.02%, respectively, and the limit of detection is 0.21 µg/ml [19]. A reliable novel method for the analysis of AA in pharmaceutical products and fruit juices has been proposed. AA is oxidized to dehydroascorbic acid (DAA) using Cu (11)-2, 9-dimethyl-1, 10-phenenthroline reagent in ammonium acetate at pH 7. The absorbance is measured at 450 nm. The method has been statistically compared with the HPLC method and gives comparable results [20]. AA reduces methyl violet to form a stable blue colored free radical ion. This property of vitamin C has been utilized for its determination by a simple and sensitive spectrometric method in foods, pharmaceutical products and biological samples. The method obeys Beer's law within the concentration range of 1.0-10 µg/ml at 600 nm [21].

A new colorimetric method for the determination of AA in pharmaceutical preparations is based upon the reaction of AA with zinc chloride salt of diazotized -1- amino-phenthraquinine (fast fed aluminium salt) in acid medium followed by development of blue color in alkaline solution. This method is found to be linear in the concentration range of 5 to 25µg/ml of AA and the absorbance measurements are carried out at the λ_{max} at 630 nm [22].

A spectrometric method involves the reaction of AA with 4-chloro-7- nitrobaenzofurazone (NBD-Cl) in the presence of 0.2 M NaOH to form blue color (λ_{max} 582 nm) in 50% (v/v) aqueous: acetone solution. This method shows good linearity in the concentration range of 5 to 20 µg AA/ml with a correlation coefficient of 0.9990. It has good accuracy for the determination of AA in the presence of DAA, rutin, salicylic acid, acetyl salicylic acid, paracetamol, caffeine, phenylepinephrine HCl and dipyrone [23].

The determination of AA in pharmaceutical fruit juices has been carried out by spectrometry. The method is based on the incubation effect in HCl and bromate methyl orange which is decolorized by the reaction product that is used to monitor the reaction spectrometrically at 510 nm. The linearity range of the calibration graph depends on bromate concentration. The limit of detection is 7.6×10^{-6} M and calibration

range is 8×10^{-6} to 1.2×10^{-3}M AA. The RSD for 8×10^{-6} to 2×10^{-5} M of AA is 2.8 and 1.7 % [24].

A spectrometric method for the determination of AA involves the oxidation of AA to DHAA with CUPRAC reagent of total antioxidant capacity, i.e/ Cu (II) – neocuproine (Nc), in ammonium acetate at pH 7, where absorbance of the formed bis (Nc) copper (I) chelate is measured at 450 nm, Flavonoids that normally interfere with CUPRAC procedure are separated with preliminary extraction as their Cu (II) chelates into ethyl acetate (EtAC). Beer Lambert law is obeyed between 8.0 x 10^{-6} and 8.0 x 10^{-5}M concentration with the equation of linear calibration curve A_{450nm} = 1.60 x 10^4 (mol dm^3) - 0.0596. The RSD for the synthetic mixture that contained 1.25 x 10^{-2} mM AA and flavonoids is 5.3 % [25].

Different simple and rapid methods have been reported for the analysis of AA by colorimetry [26-34]. A new colorimetric method has been developed for the indirect determination of AA in pharmaceutical products. The method is based on the cloud point extraction (CPE) separation and preconcentration. Fe (III) is reduced to Fe (II) by AA and than its complexation with 2-(5-bromo-2-pyridylozo)-5-diethylaminophenol is followed by its extraction into Ttiton X-114. The selectivity of the method can be increased by using EDTA as the masking agent. AA was determined in the range of 5-200 µg/L at 742 nm with the RSD of 3% [35].

Spectrofluorometry

Reaction of AA with o-phenylenediamine in the absence of the oxidant has been utilized for its determination by a simple and sensitive fluorimetric method. The fluorescence intensity is measured at the excitation and emission wavelengths of 360 and 430 nm, respectively, and the detection limit is 0.006 µg/ml [36]. Another method based on the reaction of AA with methylene blue has been reported for its determination in vitamin C tablets. The excitation and emission of the fluorescence intensity is measured at 664 and 682 nm, respectively. The concentration of AA has been found to be in the range of 3.0×10^{-7} to 6.0×10^{-6} mol/l [37]. AA is rapidly oxidized by mercury (II) chloride to DHAA which then reacts with o-phenylenediamine to give a fluorescent quinoxaline derivative. The RSD of the method was 1.9% and the detection limit was 0.02 µg/ml [38]. A strong fluorescent product is formed as a result of the reaction of AA with 2-cyanoactamide at pH 12.9-13.3. The fluorescent intensity is measured at 380 nm on excitation at 329 nm. Detection limit and concentration range of AA was 0.03 µg/ml and 0.1-50 µg/ml, respectively [39]. Leucothionine blue, a highly fluorescent compound, is formed by the photooxidation of AA sensitized by thionine blue. The method utilized AA in the concentration range of 8×10^{-7} - 5×10^{-5} mol/l. This simple and rapid method can be used for the determination of AA in fruit juices and pharmaceutical products [40]. The use of stopped-flow mixing technique can achieve rapid oxidation in this method [41]. Another kinetic-fluorimetric method has been proposed that is based on activating effect of vitamin C on the oxidation of 2-hydroxynaphthaldehyde thiosemicarbazone catalyzed by manganese (II) [42]. During the reaction of AA with 2,3-diaminonaphthalene in alkaline solution without oxidant, the addition of β-cyclodextrin has been found to enhance the fluorescence. This reaction has been used for the development of a simple and sensitive fluorimetric method. The detection limit and concentration range of AA were 8ng/ml and 0.05-600 µg/ml, respectively [43]. A study has been carried out for the determination of AA by utilizing its reactions with Ce(IV) in the presence of sulphuric acid [44], its activating effect on the oxidation of rhodamine 6G by potassium bromate catalyzed by vanadium [45], its oxidation to DAA and

then condensation with o-phenylenediamine to its quinoxaline derivative [46] and then determination in foods [47].

The simultaneous determination of thiamine and AA has been carried out by a flourimetric method by oxidation with mercury (II) to form thiochrome (TC) and quinoxaline derivative and measurement of fluorescence at the same wavelength (λ_{ex}=356 and λ_{em}=440nm). The calibration curves have been found to be linear in the concetration range of 5-100 µg/ml for thiamine and AA [48].

Mass spectrometry

AA along with its degradation products has been determined with an UV detector with a triple-quad mass spectrometer in a multiple reaction mode. All the validation parameters were within the range of acceptance. The linearity and repeatability were found to be 0.999 and 9.3%, respectively. The method has proved to be fast, simple and sensitive for AA determination [49].

Two methods have been developed for the determination of AA and lycopene and beta-carotene in vegetables and fruits by liquid chromatography coupled with mass spectrometry. In these methods separation was carried out by using two columns C_{18} and dC_{18} and mobile phase of methanol and acetic acid (70:30, v/v) for AA and methanol, tetrahydrofuran and acetonitrile (60:30:10, v/v/v) for lycopene and beta-carotene. In these methods molecular ion was selected for the quantification in selective ion monitoring (SIM) made. AA was detected by electrospray ionization probe (ESI) in negative mode and beta-carotene and lycopene were detected by chemical ionization atmospheric pressure (APCI) in positive mode. The analysis of AA is based on extraction by polytron using methanol and a mixture of metaphosphoric acid and acetic acid. For beta-carotene and lycopene extraction was carried out by tetrahydrofuran (1:1, v/v) [50].

An analytical method has been developed for the determination of the stability of AA by HPLC-triple quadrupole mass spectrometry. For the determination of AA, UV detector in sequence with triple-quad mass spectrometer in multiple reaction mode was used. In this method negative ion mode of ESI and MS-MRM transition of m/z 175-115 (quantifier) and 175-89 (qualifier) for AA were used. This method shows good accuracy, precision, linearity, LOD and LOQ. LOD and LOQ for this method was found to be 0.1524 and 0.4679 ng/ml, respectively [51].

AA and DAA in pepper, orange and lemon were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with triple quadrupole in selective reaction monitoring mode. In this method negative ion mode of ESI and MS/MS transition of m/z 173-143 and m/z 175-71 were used for AA and m/z 175-115 and m/z 175-87 for DHAA. This method is found to be accurate and precise and LODs was found to be 13 and 11 ng/ml for AA and DAA, respectively [52].

Chromatographic methods

HPLC methods

HPLC methods have extensively been used for the determination of AA. These methods have the advantage of separation of AA from other ingredients in food materials and pharmaceutical preparations followed by determination and have better accuracy than other methods. Some of these methods may take longer time than the spectrometric methods but have greater accuracy.

A new RP-HPLC method involves the simultaneous determination of five water-soluble vitamins (riboflavin, nicotinamide,

pantothenic acid, folic acid and ascorbic acid) in honey. This method shows good accuracy, precision and linearity for the determination of these vitamins [53]. For the detection of total content of vitamin C (TC) in various type of food a HPLC method has been found to be accurate, sensitive, precise and linear for the quantification of TC [54].

The separation and determination of AA, DAA, erythrobic acid and dehydroerythrobic acid, diketoglunic acid and diketogluconic acid by HPLC on a Zorbax NH- analytical column with UV monitoring at 268 nm has been achieved. It allows AA and erythrobic acid to be detected at the 25 ng level. In this method tyrosine is used as an internal standard and found that dithioreitol is effective in rapidly reducing DAA and AA [55].

Plants are known to have various mechanisms to protect themselves against oxidative stress. One of these mechanisms is non-enzymatic antioxidation of AA. For the analysis of the reduced and oxidized forms of AA in plants, an HPLC method has been developed. This method is found to be suitable for the quantification of ascorbate ion in plants [56]. A new HPLC method with columetric detection has been developed for the determination of ascorbate in RBCs of humans, wild type mice and mice unable to synthesize ascrobate. It has been found that exogenously added ascorbate is fully recovered even when endogenous RBC ascorbate is below the detection limit (25 nM). In this method 20 µL of packed RBCs ascorbate was sufficient for the determination [57].

A HPLC method has been developed for the separation and quantization of AA and DAA by RP ion-pair technique. In this method pre-column derivitization of DAA with 1,2- phenylenediamine is carried out and tridecylammonium formate or hexadecylmethylammonium bromide is used as ion-pairing agent in mobile phase. This gives excellent resolution between AA and the derivatised DAA. Analysis of the two compounds is carried out in the range of 348 nm to 290 nm [58]

A RP-HPLC method has been used to evaluate the stability of AA, ascorbyl palmitate (AP) and magnese ascorbyl phosphate (VC-PMG) in both standard solution and topical formulations after dilution with a suitable aqueous organic solvent. It has been found that AP and VC-PMG are more stable than AA [59]. A new rapid HPLC method has been involves the simultaneous determination of creatinine (CR), uric acid (UA) and AA in bovine milk samples and orange juices. In this method bovine milk samples were pretreated by protein precipitation, centrifugation and filtration. After this pretreatment separation and quantification was carried out by using a Supherisorb S5NH2 column. It has been found that in bovine milk samples 24.1-86.0 and 5.07-11.2 µg/L of UA and CR, and 212 µg/L of AA has been found [60].

A new sensitive and specific RP-HPLC method with UV detection for the determination of AA (1.25 mg/ml) and calcium gluconate (100 mg/ml) in oral solution has been developed. In this method 0.005 mol/L tetrabutylammonium hydroxide was used as an ion-pair reagent water and methanol (80:20, v/v) as mobile phase. This method was found to be linear in the concentration range of 10.0-100 µg/ml of AA with the coefficient of variance not more than 5% [61].

A new simple, rapid, sensitive and accurate ion-exclusion HPLC-UV method has been reported for the determination of AA and UA. In this method separation was carried out by isocratic elution on a HEMA-BIO1000 SB analytical column by using phosphate buffer (pH 2.4) as a mobile phase. This method was found to be linear with the regression coefficient of 0.999 and LOD for AA and UA of

1.02 and 1.42 nmol/ml, respectively, with LOQ range of 0.306-0.426 nmol/ml. The recovery range for AA and UA was found to be 92-96 and 99-100 %, respectively [62]. HPLC along with UV detector (254 nm) has been used for the determination of AA and erythroboric acid (EA). In this method Li Chrosorb-NH_2 column pretreated with ammonium mono phosphate (0.1 M) with a mobile phase of acetonitrile, acetic acid and water (81:2:11, v/v/v) has been used. This method was found to be accurate and AA and EA were detectable as low as 1.0×10^{-2} µg [63].

The determination of AA in beer has been carried out by using HPLC method with dual electrode electrochemical detection. AA is separated by using ion-suppression chromatography on d48 column and detected by the dual electrode system which consists of a graphite high efficiency electrode followed by a series of glassy amperometric electrodes. In this method beer is degraded by helium sparging in oxygen free atmosphere and then mixed with 50 ml 50 mM potassium dihydrogen orthophosphate/Na_2 EDTA (500ng/l), adjusted to pH 3 with 10 M orthophosphoric acid which acts as a mobile phase. This method is found to be linear in the concentration range of 0.5 to 2mg/l AA with the detection limit of 0.2 mg/l [64].

AA, isoascorbic acid (IA) and UA in human plasma are directly determined by paired ion RP-HPLC. An ultra sphere ODS (C18) column is used with a pH of 5.25 of mobile phase that contains 0.04 M sodium acetate, 0.005 M tetrabutylammonium phosphate and 0.2 mg/ml of disodium ETDA. Plasma samples are preserved with an equal volume of 10 % metaphosphoric acid and are diluted 10 folds with the mobile phase and filtered through 0.24 micron filters. In this method less than 0.25 µg of each component can easily be detected [65].

The determination of DAA in biological samples commonly involves indirect measurements by subtraction of the amount of AA concentration from that of total AA which is formed after the reduction of DAA. AA concentration is dependent upon proper sampling, handling, and quantitative reduction of the compound and accurate quantification of both AA and total AA. It has been found that tris [2-carboxyethyl] phosphine hydrochloride (TCEP) is a reliable alternative for the commonly used reducing agent dithiothreitol (DTT). TCEP gives better reduction of DAA at low pH as compared to DTT. The coefficients of variation for the complete assay within and between days for all analysis are less than 1.5 and 3.5% [66]. Some of the HPLC methods which have been used for the determination of AA are reported in Table 2.

Thin Layer Chromatography (TLC)

A TLC method has been developed for the determination of AA in beverages. The AA concentration was measured after separation by silica gel TLC. The concentration of AA is visually determined by the time in autocatalytic reaction for AA spot turns to the same yellow color of the background and disappears after spraying with 3, 6-dihydroxyxanthane solution. It has been found that there is good linear relationship between the end time the induction period and concentration of AA for concentration in the range of 5.0-2.0 mM with the linear regression of 0.9944. The %RSD for AA in 3 beverages was less than 5 % and recovery was found to be in the range of 97-110% [78].

AA in micronutrients is readily determined by using 2,4-dinitrophenyl hydrazine method along with separation with TLC. Glass fiber disc was used for condensation reaction for 5 min at 100 oC. Purification of AA-hydrazone complex was carried out by separation through TLC plates. An aqueous solution of 65% acetic acid was used to dissolve this complex and give maximum absorbance at 500 nm. Minimum

quantification level for DAA was found to be 0.4 µg and macrodetermination of AA can be improved by using 65% acetic acid as a solvent for hydrazine [79]. AA was also determined through TLC in different materials and these methods have shown good applicability [79-82], also in combination with other vitamins [83, 84], drugs [85-88] and in comparative studies [89].

A method for the semiquantative determination of water-soluble vitamins such a thiamin hydrochloride, riboflavin, pyridoxine, choline chloride, p-aminobenzoic acid, cyanocobalamin and ascorbic acid has been reported. This method involves circular spots or rings on chromatographic apparatus and is based on the sensitivity and specificity of spot reactions together with comparison of the color intensity of circular spots. The accuracy of this method was found to be ± 5% [90].

Titrimetric Methods

Potentiometry

Many workers have analyzed AA in neat solutions [91-93], mixtures [94] and pharmaceutical products by potentiometric method [95, 96]. In the ascorbate oxidation with permagnate, a ligand-free tridodecylmethylammonium chloride (TDMAC)-based polymeric membrane ion selective electrode (ISE) is used as the basis for the determination of AA by potentiometric method. The ISE potential increases with the consumption of permagnate and the membrane can be used continuously and for reversible detection with the detection limit of 2.2×10^{-7}M [97].

For the determination of AA in urine and pharmaceutical dosage forms, a simple, sensitive and reliable method has been proposed that is based on the pulse perturbation technique. The response of the Bray-Liebhafsky (BL) oscillatory reaction to the perturbation by different concentrations of vitamin C is followed by Pt electrode. The limit of detection has found to be 0.009 micromol and the linear relationship has been determined between 0.01-1.0 micromol [98]. Another simple method for the rapid estimation of AA in pharmaceutical products has been reported. This indirect potentiometric titration is based on treating AA with iodine using copper based mercury film electrode as an indicator. The method has shown good precision and accuracy and the results have been compared with those of BP methods using F and t-statistical tests of significance [99]. AA is reduced by IO3- and the detection is carried out by ion selective electrode for iodide. By this method AA can be determined in the range of 7.5-15.0 mmol/l. The %RSD of the method has been found to be 1 [100].

An electrode constructed by pre-treating silver tube with mercuric chloride solution and iodide solution in flow injection analysis has been used as a potentiometric sensor for the analysis of AA and glutathione. The method has also been used for the determination of AA in pharmaceutical preparations [101]. In another method the electrode has been prepared by incorporating iron phthalocyanine into carbon-paste mixture which showed catalytic properties for the electrooxidation of AA. The detection limit has found to be 5×10^{-7} M and the recovery was 97.2-102.4%. The method has shown fast response time and good stability [102].

The determination of AA through potentiometric method has been carried out by using the semi-automatic direct titration. In this method potassium iodide, strongly acidic solution is used as titrant with iodide ion-selective electrode for end point detection. The determination of AA, thiourea and thiosulfate in this titration is in the range of 0.15-1.5 mg, respectively [103].

Several studies have been conducted for the

determination of AA in neat solutions [104-106] pharmaceutical products [107-110], foods [111-113] and in the presences of other vitamins [114, 115] utilizing potentiometric methods.

Redox Titrations

Iodometric titration has been utilized for the determination of AA in vegetables. It was extracted by using 2% oxalic acid, 10% HCl, mixture of 2% oxalic acid and 4% acetic acid. This simple, rapid and sensitive method showed a recovery of 102.9% [115].

AA has been determined in the presence of a variety of potentially chemicaly and physicaly interfering species in commercially prepared liquid diets. In this method the titrant and indicator used are 2,6 dichlorophenolindophenols, and iron (II), copper (II), cysteine, glutathione, sulfate and tin (II). These reagents which were used in this titrimetric determination are not interfering in the assay of AA [116].

AA is also oxidized to DAA by bromine and excess of potassium bromide is added to acidify the solution of the sample. Then this solution is titrated with standard potassium bromide to the first permanent appearance of excess bromine and this excess is then determined iodometrically with standard sodium thiosulphate [118].

AA is used as a weighable compound for the standardization of iodine solution in an analytical experiment. In this method iodine reacts with AA resulting in the conversion of AA into DAA. The redox titration end point is determined by the excess of iodine that complexes with starch and gives blue- violet color. This method is accurate and precise and with some advantages, i.e., saving time and avoiding mistakes due to solution preparation. The colorless AA solution gives a very clear and sharp titration end point with starch [119].

In another method, the redox indicator, ferroin, tris (1, 10-phenanthroline) iron (II), is incorporated into the perflurosulfonated cation- exchange membrane Nafion together with optical fibers. In this method photodiode, light emitting diode has been used for the construction of redox optical sensor used as optode . This is then incorporated in the flow-through cell of computer-controlled sequential injection (SI) system and used for the determination of AA. By this method the concentration of AA can be determined in a variety of commercial pharmaceutical preparations and results are similar to those obtained by iodometric titration [121].

A study has been carried out on two sequential injection titration systems with spectrometric detection of AA. The detection of AA with the first system was based on redox reaction between AA and permanganate in acidic medium. These determinations tend to decrease the color intensity of permanganate monitored at 525 nm. It was observed that there is a linear dependence of peak area obtained by AA concentration up to 1200 mg/l. The RSD (n=11) of the determination of 400 µg/l of AA was 2.9%. The second method is for the determination of acetic acid, which was based on acid-base titration with sodium hydroxide using phenolphthalein as an indicator. The RSD for (n=1) of the samples were 4.8% [122].

CONCLUSION

The ascorbic acid molecule possesses a redox system which enables its determination by titrimetric methods. In addition to this it posses a –CH=CH-C=O system and which enables its determination by UV spectrometry and on colour development by visible spectrometry. Other methods of determination include fluorimetry, LC-MS and electrochemical methods. All these methods have been applied to the determination of AA in foods, pharmaceuticals and biological samples.

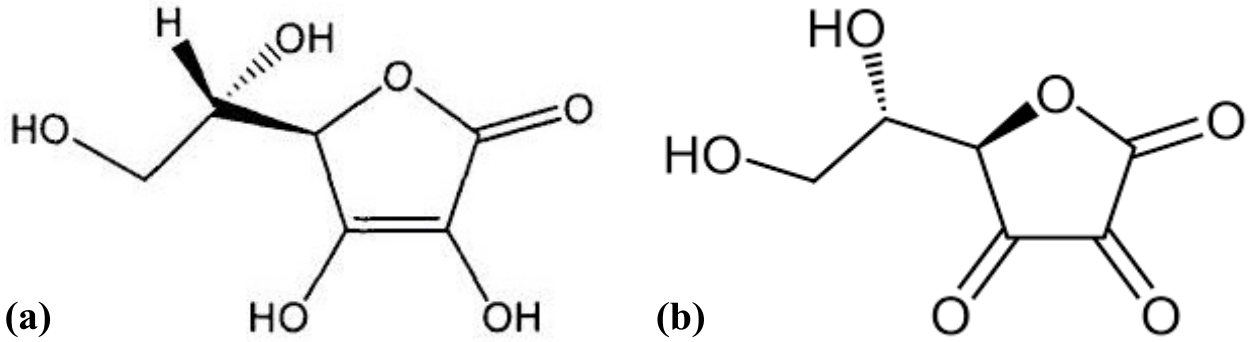

Fig. 1. Chemical structures of ascorbic acid (a) and dehydroascorbic acid (b)

Table 1. Physicochemical properties of Ascorbic acid [11]

Molecular formula	$C_6H_8O_6$
IUPAC name	(5R)-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxy furan 2(5H)-one
Molecular weight	176.1
Density	1.65 g/cm^3
Melting point	190°C decomposes
Appearance	White to slightly yellow powder or crystals
Odor	Odorless
Taste	Pleasant, sharp, acidic
Solubility (g/L)	
In water	330
In ethanol	20
In glycerol	10
In propylene glycol	50
In diethyl ether, chloroform, oil, petroleum ether	Insoluble
UV Spectrum Absorption maximum [A(1%, 1cm)]	
pH 2.0	245 nm [695]
pH 7.0	265 nm [940]
pKa	4.17 (at 25°C)
Log P	-1.85
pH	3 (5 mg/ml), 2 (50mg/ml)

Table 2. Analytical parameters for the assay of ascorbic acid by HPLC methods

Material	Technique	Column	Mobile phase	Flow rate ml/min^{-1}	Detection nm	Conc. range µg/ml^{-1}	Reference
α arbutin, β arbutin, kojic acid, nicotinamide, resorcinol, ascorbic acid, hydroquinone, 4-methoxyphenol and 4-ethoxyphenol	HPLC-UV	C-18	20 mM NaH_2PO_4 containing 10% methanol at pH 2.3	-	-	-	67
Ascorbic acid, aminothiols and methionine	RP-HPLC	C-18	Methanol – 0.05% trifluoroacetic acid solution	1.5	-	-	68
Retinol, retinyl palmitate, βcarotene, α tocopherol and ascorbic acid	HPLC	Intersil ODS3	Methanol-tetrahydrofuran-water	1.5	270	-	69
Vitamin B1, B2, B3, B5, B6, B9, C in vitamin and mineral tablets	HPLC with diode array detector	C-18	Phosphate buffer – acetonitrile (95:5, v/v)	0.5	275	-	70
Vitamin C and other B complex vitamins	RPLC	C-18	10 mM potassium dihydrogen phosphate buffer (containing 3 mM sodium hexane-1- sulfonate, adjusted to pH 2.8 with o-phosphoric acid) and methanol	1.0	274	-	71
Ascorbic acid, citric acid and amines in citrus	HPLC	Xbridge C-18	3 mM phosphoric acid	1.0	223, 254	9.8 ng	72
Vitamin C, paracetamol, chlorphenamine maleate and chlorogenic acid in vitamin C tablets	RP-HPLC	Sinochrom ODS-BP	0.05 mol/L KH2PO4 (pH 3.0, containing 1% triethylamine and acetonitrile; 75:25, v/v)	-	260, 326	-	73
Ascorbic acid and dehydroascorbic acid in plant materials	HPLC	Sperisorb S5ODS	Methanol:water (1:3, v/v) containing 1mM hexadecyltrimethyl ammonium bromide and 0.05% (w/v) sodium dihydrogen phosphate monohydrate (pH adjusted to 3.6 by adding orthophosphoric acid)	1.0	248	-	74

Material	Technique	Column	Mobile phase	Flow rate ml/min^{-1}	Detection nm	Conc. range µg/ml^{-1}	Reference
Ascorbic acid in foods and multivitamin products	HPLC	Bondapak C18	2.0×10^{-3} M NH_4OH, pH adjusted to 5 with 1% formic acid	3.0	254	-	75
Ascorbic acid and malondialdehyde in human serum	HPLC-UV	Wakosil II 5C18 RS	82.5:17.5 (v/v) 30 mM mono basic potassium phosphate (pH 3.6) - methanol	1.2	245	-	76
Ascorbic acid and dehydroascorbic acid	HPLC	Bondapak NH2	50:50 (v/v) methanol-0.25% KH_2PO_4 buffer (pH 3.5)	0.8	244	-	77

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