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Research Article

A Comprehensive Study of Stress Degradation Pathways of Ascorbic Acid to Standardize Flavonoids-Related Impurities Derived from Ascorbic Acid

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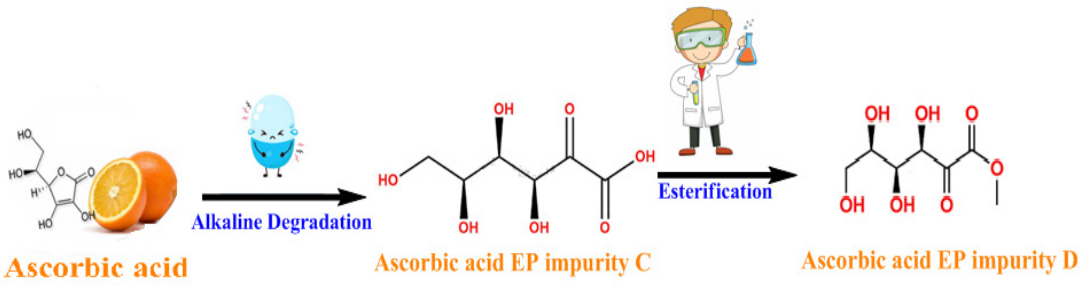
ABSTRACT

Ascorbic acids are gaining popularity as functional food or health supplements owing to their antioxidant property. Ascorbic acid API or antioxidant ingredients were subjected to force degradation studies under various hydrolysis conditions as per the International Council for Harmonisation (ICH) guidelines, i.e., acidic, alkaline, thermal, oxidative, and photolytic. The degraded samples were analyzed using a compatible self-developed high performance liquid chromatography (HPLC) method. The drug was found to be highly sensitive to alkaline environments and exhibited significant degradation. The drug has also shown degradation when exposed to oxidative stress. The drug was found to be quite stable in acidic, photolytic, and thermal conditions. The major degraded product obtained in alkaline condition was isolated, purified and characterized as (3S, 4R,5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid with a molecular mass of 194.14 g/mol and a molecular formula of $C_6H_{10}O_7$. This compound is a pharmacopeial impurity (EP impurity C) of the drug. This compound was then converted to its ester form to further ascertain its structural configuration. The ester was characterized as (3S, 4R, 5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate (EP impurity D) with a molecular mass of 208.17 g/mole and a molecular formula of $C_7H_{12}O_7$ The compounds were characterized with nuclear magnetic resonance (NMR) and mass spectroscopic technique. When ascorbic acid was subjected to oxidative stress, a water-soluble compound was obtained as the major degradant. Its isolation, identification and characterization are under study. A mechanism for the formation of (3S, 4R, 5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid as the major degradant in alkaline stress was proposed.



Graphical Abstract

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INTRODUCTION

In the last few decades, studies of compounds having antioxidant properties have gained popularity as it promotes health and well-being.[1-2] Antioxidants help in stabilizing, inhibiting, deactivating, and scavenging free radicals produced in our body, thus protecting us from oxidative damage. Flavonoids demonstrate rich antioxidants and safeguard cellular damage against reactive free radicals produced in human bodies during various metabolic processes.[3-6] Studies showed that most of the flavonoids get degraded to phenolic acid, but they still possess a radical scavenging potential.[7] Flavonoids such as ascorbic acid, vitamin P, vitamin B12, beta carotene, etc. exhibit significant antioxidant properties.[8-11] Ascorbic acid, (*R*)-5-((*S*)-1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5*H*)-one (Fig. 1) is a natural water-soluble vitamin (Vitamin C) and plays a very important role in many pharmacological activities in the human body, and thus it is used as a core ingredient in the formulation of various cosmetic and pharmaceutical products.[12] Oxidation in ascorbic acid is a two-step phenomenon.[13] Firstly, it dissociates into monodehydroascorbate and subsequently into its dehydroascorbate. Ascorbic acid-loaded poly(lactic-co-glycolic) acid nanoparticles are found effective against oral cancer.[14] Being cheap and readily available, it is often used as a supplement to boost immunity and as a food preservative.[15]

The chemical stability of pharmaceutical molecules is a major problem as it impacts the safety and efficacy of the drug over time under the influence of various environmental conditions.[16-17] It ensures drug stability at a very early stage of drug development.[18-19] Forced degradation is a process that involves the degradation of drug products and drug substances under less favorable conditions.[20-21] This study also helps us to know the degradation pathway.[23] It helps us to know the various impurities developed during the storage of drugs under varied environmental conditions. The stability of ascorbic acid depends largely on physicochemical conditions[24] and therefore its force degradation studies are quite necessary to improve its preservation and storage.[25] Ascorbic acid is reported to degrade under several parameters, including temperature, pH, time, acidic, basic, anaerobic condition, and aerobic conditions.[26]

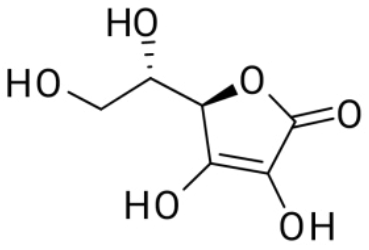

Fig. 1: Structure of 5-(1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5*H*)-one (Ascorbic acid)

Goshima *et al.*'s kinetic analysis of the ascorbic acid breakdown process in acidic conditions produced two epimeric intermediate 3,6-dihydroxy-3a-methoxytetrahydrofuro[3,2-*b*] furan-2(5*H*)-one, which following subsequent therapy provided 2-(furan-2-yl) derivatives of -2-oxo acids. The degradation was carried out in methanol in the presence of boron trifluoride etherate under non-oxidative conditions.[27] Herbig *et al.* and Sarturi *et al.* reported that the rate of ascorbic acid degradation is significantly dependent on temperature, being rather stable between 35 to 40°C and rapidly degrading between 60 to 80°C. Herbig and his group did a systematic and quantitative study and found that concentration has no impact on degradation. Sarturi used a kinetic model based on the transition state to study the acid during degradation.[28,29] According to Pilarski *et al.*'s potentiometric titration research on the ascorbic acid degradation behavior in aqueous solution, the ascorbate ion formed during degradation is efficiently protected against oxidation by the thiocyanate ion. They suggested that a paraffin protection layer may be used for its storage.[30] In a study by Marfil *et al.*, greater drying time and high temperatures led to more deterioration of ascorbic acid-containing products while they were being preserved.[31] A spectrophotometric analysis of ascorbic acid degradation published by Nasheed *et al.* found that heat degraded the acid more than UV or alkaline environments.[32] An article published by Avdeeva *et al.* stated that the degradation of ascorbic acid in anaerobic conditions or the presence of peroxides increased by many folds in the presence of a metal (Cu)-binding substances. The study focuses on the redox-active properties of copper-binding compounds in the oxidation degradation of L-ascorbic properties.[33] Using pH as a parameter, Yuan *et al.* found four major compounds from ascorbic acid degradation: 2-furoic acid, 3-hydroxy-2-pyrone, furfural, and an unidentified compound. An high performance liquid chromatography (HPLC) method was employed to study the degradation of the acid at different pH levels.[34] Tatum *et al.* claimed to obtain 15 degraded products of ascorbic acid. They also reported the direct effect of temperature on its degradation with the lowest degradation obtained at 0°C.[35]

Ascorbic acid was degraded under several parameters, including temperature, pH, time, acid, base, anaerobic, and aerobic conditions[27-35] over the last two decades, and it is reported that it gets degraded easily, but the resultant products are difficult to isolate. It was also observed that none of the degraded compounds obtained were pharmacopeial impurities. Some of the studies performed on ascorbic acid in the past few years have been summarized in Table 1.

In this present work, we have studied the force degradation behavior of ascorbic acid in acidic, alkaline, oxidative, photolytic, and thermal conditions as per ICH guidelines.[36-40] The major degradant products were isolated and characterized by their spectral data.

Table 1: Summarized results of the study

S. No.	*Author (Year)*	*Study performed*	*Inference*	*References*
1	Goshima *et al.* (1973)	Kinetic analysis of Acid degradation	Formation of 3,6-dihydroxy-3a-methoxytetrahydrofuro[3,2-*b*] furan-2(5*H*)-one, which further gave 2-(furan-2-yl) derivatives of -2-oxo acids.	[27]
2	Herbig *et al.* (2017)	Stress degradation using temperature	Stable at ambient temperature (35–40°C), and less prone to degradation	[28]
3	Sarturi *et al.* (2012)	Stress degradation using temperature	Higher temperature (60–80°C) is prone to produce rapid degradation	[29]
4	Pilarski *et al.* (2003)	Potentiometric titration of aqueous solution of ascorbic acid	The ascorbate ion formed during degradation is efficiently protected against oxidation by the thiocyanate ion	[30]
5	Marfil *et al.* (2008)	Loss on drying	Greater drying temperatures led to more deterioration of ascorbic acid-containing product during preservation	[31]
6	Nasheed *et al.* (2015)	UV Absorption study of various degradation products	Acidic degradation performed at higher temperature are more likely to produce degradation products than alkaline degradation or photolytic degradation	[32]
7	Avdeeva *et al.* (2020)	Degradation studies under various conditions	The presence of (Cu)- binding substances increases the degradation of ascorbic acid to many folds in anaerobic or peroxide conditions	[33]
8	Yuan *et al.* (1998)	Degradation of ascorbic acid in different pH	Found four major compounds from ascorbic acid degradation: 2-furoic acid, 3-hydroxy-2-pyrone, furfural, and an unidentified compound	[34]
9	Tatum *et al.* (1969)	Various degradation Pathways	Obtain fifteen degraded products of ascorbic acid in different degradation parameters	[35]

This study will help the research community understand the acid in a better way and help in selecting suitable prevention methods for its storage and improving self-life. As the study showed ascorbic acid is vulnerable to alkaline and oxidative stress, it may be stabilized by treatment with some other reagents. As the major degraded product of ascorbic acid in alkaline stress is a pharmacopeial impurity, its prevention is of utmost importance to reduce relevant risk assessments.

Materials and Methods

Chemicals and Reagents

Chemicals and reagents were procured from Sigma Aldrich, TCI, and BLD Pharma and were of synthetic grade. The solvents used were of commercial grade. Reaction procedures were optimized on the SJI magnetic stirrer.

Instrumentation

The reactions were monitored by TLC using pre-coated aluminum plates procured from Merck silica gel 60F-254 using the UV visualization technique, Ninhydrin reagent, and iodine vapors. The melting points of each of the compounds obtained were recorded by the open capillary method and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) and DMR were recorded on a "Bruker Advance" spectrometer at 400 MHz frequency in DMSO D6 in the presence of TMS as an internal standard (chemical shift in ppm) (DMR refer Hydrogen-deuterium exchange ^{1}H-NMR). The abbreviations s, d, dd, t, and m stand for singlet, doublet, double-doublet, triplet, and multiplet, respectively. Chemical shift (δ, ppm) and coupling constants (Hz) are reported in a standard fashion. Mass spectra were obtained from the MD SCIEX API3200 liquid Chromatography with tandem mass spectrometry (LC-MS-MS) system equipped with an electrospray ionization technique. HPLC was performed using the Agilent 1100 series and the C18 column. The purification of synthesized compounds was done by the BUCHI C-700 Prep-chromatographic technique.

Stability data and force degradation study of a drug is very crucial for its regulatory approvals. The Food and Drug Administration (FDA) and International Council for Harmonisation (ICH) have been mandated to highlight stress degradation findings while reporting any new drugs. This helps in understanding the behavioral pattern of the drug over time and in a different environment. The effect of chemical reactions which may occur with the API is evaluated. Hydrolysis of antioxidant molecules as a flavonoid may occur in an acidic or basic environment, and oxidation in the presence of reactive oxygen species. These reactions are performed to understand the chemical stability of the API. A chromatogram of the resultant samples is shown.

Optimization of Degradation Studies

Ascorbic acid was initially treated with an alkaline solution to evaluate the forced degradation behavior of the antioxidant molecule. The degraded products obtained were isolated and identified. The antioxidant molecule was further subjected to acidic, thermal, oxidative, and photolytic degradation.

Samples were withdrawn from the stress study reaction

at regular intervals and analyzed using thin layer chromatography (TLC) and HPLC. For HPLC analysis 10 µL of sample was injected.

Optimization of Alkaline Degradation

Alkali degradation study

One mol of ascorbic acid was suspended in 10 volumes of 0.1 N aq. sodium hydroxide solutions and the reaction was stirred at room temperature for 72 hours. After 72 hours, the test sample was withdrawn and analyzed, and minute degradation was observed (1.4% by HPLC). Then the concentration of sodium hydroxide was increased to 0.2 N and the reaction continued for 2 hours at room temperature. But when TLC monitored the progress of the reaction, it was negligible, hence the temperature of the reaction was elevated to 75°C and stirred again for 72 hours. At this stage, the test sample was withdrawn for HPLC analysis which showed the formation of a 4.89% degraded product. Finally, the concentration of sodium hydroxide was increased to 6 N and the reaction continued for 72 hours. The chromatogram of the sample obtained after stirring the API for 72 hours in 6 N sodium hydroxide solution at 75°C, gave gave satisfactory degradation of 14.60%. The reaction was left undisturbed for 2 hours to attain room temperature and later acidified to obtain a pH in the range of 2 to 3. The organic compound was isolated using ethyl acetate and purified using flash chromatography. Finally, the compound was recrystallized in methanol to obtain a pure compound. It was then characterized by spectral and chromatographic analysis to confirm its structural configuration which was found to be (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid. The melting point was determined and matched with the reported data for conformational analysis.

Conversion of (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxo hexanoic acid to (3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxo hexanoate

One mmol of the isolated compound (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxo hexanoic acid) was suspended in 2 volumes of methanol, and a catalytic amount of conc. sulfuric acid was added to it. The reaction continued at room temperature for 4 hours. The reaction was monitored using TLC, and an additional spot was observed when ninhydrin was stained. At the end of the reaction, the mass was neutralized using aq. sodium bicarbonate. The organic compound was extracted using ethyl acetate. It was then purified using column chromatography to obtain a white, crystalline solid. The same reaction was repeated under high pressure, and similar results were obtained. The synthesized compound was further characterized using spectral data.

Acidic degradation study

To evaluate the behavior of ascorbic acid's breakdown in an acidic environment, 1 gram of the API was added to a 10 mL solution of 0.1 N of hydrochloric acid, and the mixture was stirred for 72 hours at room temperature. The material was TLC checked after 72 hours, and no discernible change was observed. The mixture was then heated to 40 to 50°C and stirred again for 72 Hours. However, there was no modification in the TLC or HPLC chromatogram. The concentration of hydrochloric acid was increased to 0.2 N, and the reaction continued for 72 hours. The analysis of test samples withdrawn at this stage showed no changes. Finally, the concentration of hydrochloric acid was increased to 6N, and the resulting mixture was then agitated for 72 hours at 40 to 50°C. The TLC was monitored, and no notable change was found in any of the reaction mixtures. HPLC analysis also showed that the degradation process was negligible. Hence, it could be concluded that ascorbic acid is quite stable in acidic environments.

Thermal degradation study

The application of heat during thermal processing may degrade the acid. To evaluate the role of temperature in the force degradation of ascorbic acid, 1-gram of the API was heated at 105°C for 72 hours. No significant change was observed in the TLC or HPLC chromatogram. The temperature of the oven was kept at 125 and 150°C, and finally 175°C with 1-gram of ascorbic acid (API) for 72 hours. The samples failed to give any significant spots while TLC was monitored or HPLC was examined. As a result, we can conclude that ascorbic acid has high thermal stability.

Photolytic degradation study

Exposure to light could be a significant factor that can lead to degradation of ascorbic acid. Hence this study was undertaken. Ascorbic acid API (200 mg) was exposed to 254 and 365 nm ultraviolet radiation at first for 48 hours as a layer (1-mm thickness) spread in an open petri dish to study its photolytic degradation pattern. Test samples were withdrawn and dissolved in methanol (0.2 mg/mL). In the TLC or HPLC assessments, no appreciable change was found. The exposure time was initially increased to 96 hours and then increased to 10 days, yet no degradation was obtained. Then, a solution of ascorbic acid API (200 mg) in 10-volume methanol was subjected to ultraviolet radiation of 254 and 365 nm in an open petri dish initially for 48 hours and then increased to 96 hours, 7 days, and 10 days. No significant change was observed in TLC or HPLC studies. Hence, it was concluded that ascorbic acid is quite stable at 254 and 365 nm when exposed for several days.

Oxidative degradation study

Oxygen availability is one factor that could contribute to degradation. Hence, we forced the oxidative breakdown of ascorbic acid in hydrogen peroxide to study the process. Initially, 1-gram of the API was dissolved in 10 volumes of hydrogen peroxide (30%) to obtain a turbid

mixture, which eventually turned into a clear solution upon stirring at room temperature for 24 hours. When TLC was monitored after 4 hours, an additional spot was observed at about Rf 0.2. The additional spot is visible in ninhydrin and iodine stains but is UV-inactive. In HPLC, at 2.6 RT the degraded product shows a peak. The product was attempted to be isolated, which is found to be highly soluble in water. To enhance the concentration of the degraded compound, 10 grams of the API were dissolved in 10 volumes of hydrogen peroxide (30%) and stirred for 24 hours at room temperature. At the end of the reaction, the solvent was completely removed by distillation to obtain a jelly-like substance. However, due to its high aqueous solubility, extraction and isolation are quite challenging. Studies are going on to isolate and characterize the degraded compound.

Results and Discussion

Alkaline Degradation

From the degradation profile obtained, ascorbic acid was degraded when 1-gram of the API was dissolved in a 6N sodium hydroxide solution (10 volumes) and stirred for more than 72 hours. The degradation was monitored through TLC and HPLC, to obtain a reasonable conversion. The degraded compound was obtained after neutralizing the reaction mass using acid and then extracted with ethyl acetate. The compound was isolated using flash chromatography (in 87.85% yield) and then crystallized in methanol (95.07%) for its analytical and spectral studies. The compound was characterized as (3*S*,4*R*,5*S*)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid from its analytical and spectral data (^{1}H-NMR, DMR, MS) shown in Figs 2-3 and 4. The degraded product was studied for HPLC purity and found to be >95% pure. A detailed result of the major degradation product is discussed below and HPLC chromatogram are shown in Figs 4 and 5.

(3S,4R,5S)-3,4,5,6-Tetrahydroxy-2-oxo Hexanoic Acid) (Ascorbic Acid EP Impurity C)

(Beige colored solid); mp: decomposed at 124°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.31 (1H, s, C_6-H), 3.42 (1H, m, C_4-H), 3.71 (4H, s, C_4-OH, C_5-OH, C_5-H, C_3-OH), 3.89 (1H, m, C_6-OH), 4.06 (1H, m, C_6-H), 4.33 (1H, s, C_3-H). DMR (400 MHz, DMSO-d_6) δ: 3.31 (1H, dd, C_6-H, J_1 = 10.4 Hz, J_2 = 6.0 Hz), 3.40 (1H, dd, C_4-H, J_1 = 10.6 Hz, J_1 = 7.8 Hz), 3.71 (1H, m, C_5-H), 4.05 (1H, d, C_6-H, J = 2.2 Hz), 4.35 (1H, s, C_3-H). MS (ESI): m/z = 193.4 ([MH$^-$]). 95.07 % HPLC purity.

The proton NMR spectra are found to be similar concerning the number of peaks. In ^{1}H-NMR, one of the protons at C_6 gave a singlet at 3.31 ppm while the other appeared at 4.06 ppm as another singlet. The proton at C_4 gave a multiplet at 3.42 ppm. The proton in C_5, and the hydroxyl group of C_4-OH, C_5-OH, C_3-OH, and C_5-OH resonated to give a singlet at 3.71 ppm. At 3.89 ppm a multiplet was obtained for the hydroxyl proton at C_6. The C_3 proton was

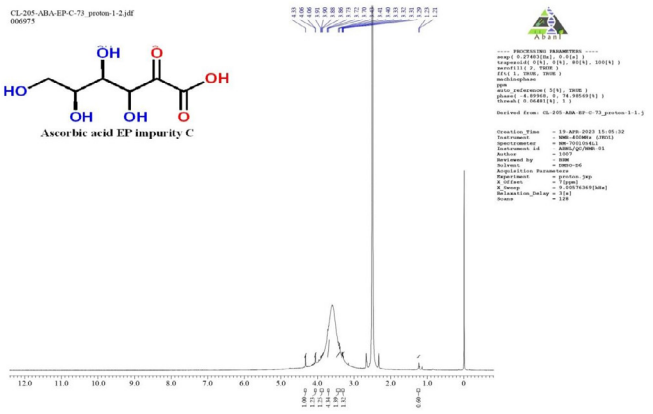

Fig. 2: ^{1}H-NMR spectra of (3*S*,4*R*,5*S*)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid

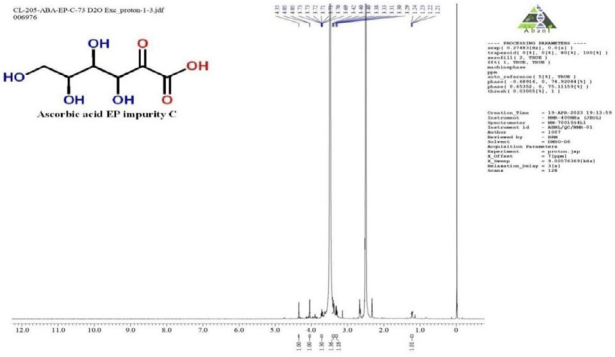

Fig. 3: DMR spectra of (3*S*,4*R*,5*S*)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid

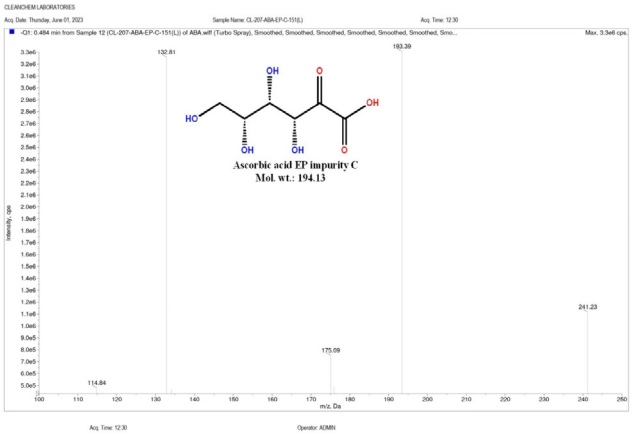

Fig. 4: Mass spectra of (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid

de-shielded to give a singlet at 4.33 ppm. In the DMR, all the hydroxyl protons were masked, and the remaining protons were obtained. At 3.31 ppm C_6 proton gave a double doublet due to resonance between the other C_6 proton and the adjutant C_5 proton. C_4 has also shown a double doublet at 3.40 ppm due to the two adjutant protons at C_5 and C_3. The proton at C_5 appeared as a multiplet at 3.71 ppm. At

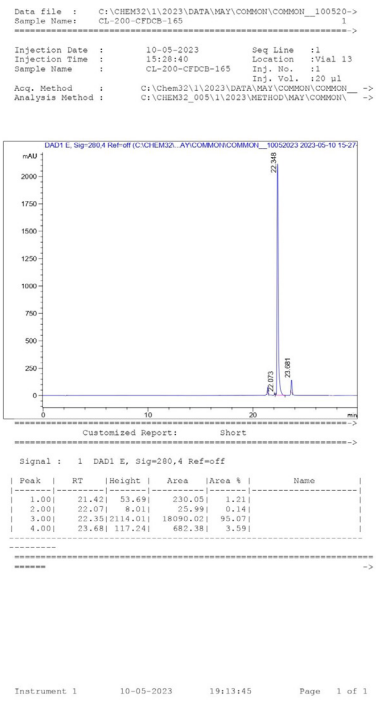

Fig. 5: HPLC Purity of (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid obtained after crystallization

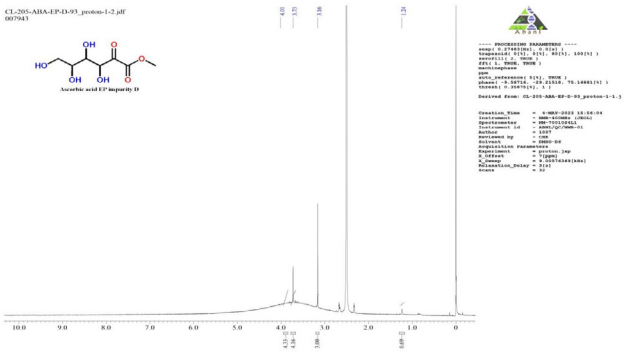

Fig. 6: NMR of ((3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate)

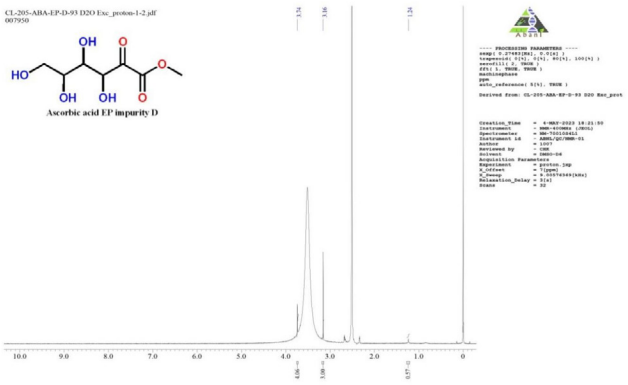

Fig. 7: DMR of ((3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate)

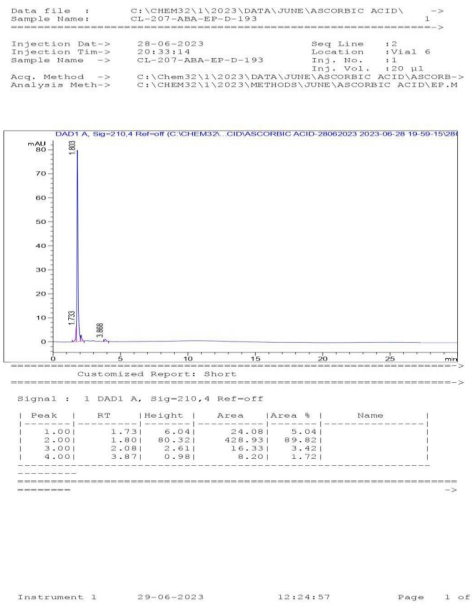

Fig. 8: HPLC purity of the synthesized compound ((3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate)

4.05 ppm, a doublet was obtained for C_6. While the proton at C_3, appeared as a singlet at 4.35 ppm. The molecular weight of the compound is 194.13. Its mass spectrum gave a signal at 193.4 m/z for MH^-.

To further ascertain the formation of (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid as the major degraded product of ascorbic acid degradation in an alkaline environment, the compound was converted to its respective methyl ester. The degraded compound (purified to 95.07%) was reacted with methanol in the presence of H_2SO_4 under conventional stirring as well as an autoclave. To our pleasure, the desired product (3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate) was obtained in both reaction conditions, which is confirmed from its analytical and spectral data. ^{1}H-NMR and DMR are shown in Figs 6 and 7, respectively. HPLC chromatogram and mass spectrum of the synthesized compound are presented in Figs 8 and 9.

(White colored solid); mp: 142-143 ^{o}C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.24 (1H, s, C_6-H), 3.16 (3H, s, OCH_3), 3.73 (4H, s, C_6-H, C_4-H, C_5-H, C_3-H), 3.89 (4H, m, C_3-OH, C_4-OH, C_5-OH, C_6-OH). DMR (400 MHz, DMSO-d_6) δ: 1.24 (1H, s, C_6-H), 3.16 (3H, s, OCH_3), 3.74 (4H, s, C_6-H, C_4-H, C_5-H, C_3-H). MS (ESI): m/z = 209 ([MH^+]) 207 ([MH^-]). 89.82 % HPLC purity. In the NMR spectra, the expected signal was obtained at their calculated chemical shift. One of the C_6 protons was obtained at 1.24 ppm while another was obtained at 3.73 ppm. The methoxy group gave a singlet at 3.16 ppm. At 3.73 ppm the protons at C_6, C_4, C_5, C_3 gave a singlet. While the proton at the hydroxyl group (C_3-OH, C_4-OH, C_5-OH, C_6-OH) gave a multiplet at 3.89 ppm. In DMR, at 1.24 ppm C_6 showed a singlet. At 3.16 ppm the singlet for the methoxy group was obtained. At 3.74 ppm, the protons at C_6, C_4, C_5, C_3 merged to give a singlet. In the mass spectrum, both Q+

Table 2: Summarized results of the study

Degradation parameter	*Reaction condition*	*Results*
Alkaline	Stirred in 0.1 N NaOH for 72 hours at room temperature	1.40% ascorbic acid EP impurity C formed
	Stirred in 0.2 N NaOH for 72 hours at 70-80 °C	4.89% ascorbic acid EP impurity C formed
	Stirred in 6 N NaOH for 72 hours at 70-80 °C	14.60% ascorbic acid EP impurity C formed
Acidic	Stirred in 0.1 N HCl for 72 hours in 40-50 °C	API remained stable without any degradation
	Stirred in 0.2 N HCl for 72 hours at 40-50 °C	
	Stirred in 6 N HCl for 72 hours in 40–50°C	
Thermal	Heated up to 175°C for 72 hours	API is thermally stable
Photolytic	Dry exposure at 254 and 365 nm for 10 days	Stable when exposed to ultraviolet radiation
	Alcoholic solution of API exposed to 254 and 365 nm for 10 days	
Oxidative	Stirred in 10 volume hydrogen peroxide for 24 hours	Degradation observed, the degraded compound is water-soluble

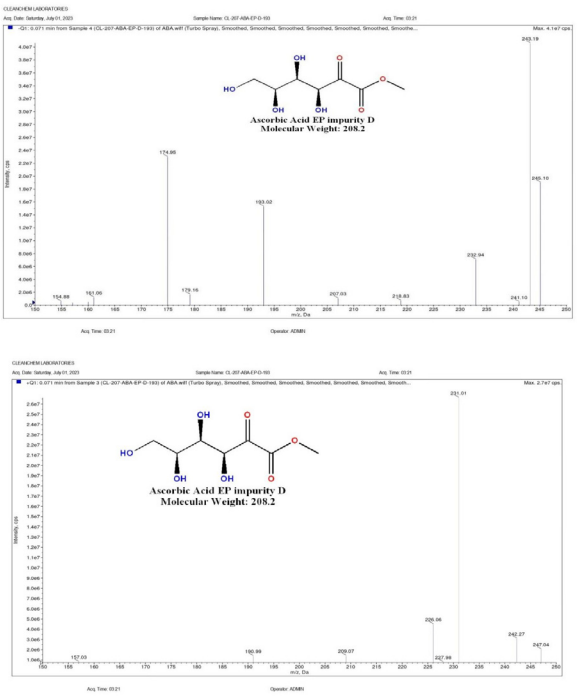

Fig. 9: Mass spectra of ((3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate)

(209) and Q-(207) modes showed a signal matching the molecular weight of the compound (Fig. 9).

Acidic Degradation

In 1 mol of ascorbic acid was added to 10 volumes of HCL solution and stirred up to 72 hours at 60 to 70°C. The chromatographic studies of the sample showed no prominent degradation.

Thermal Degradation

One gram of ascorbic acid was weighed, dried and heated in an oven at 175°C for 72 hours. No chromatographic separation was achieved from the samples.

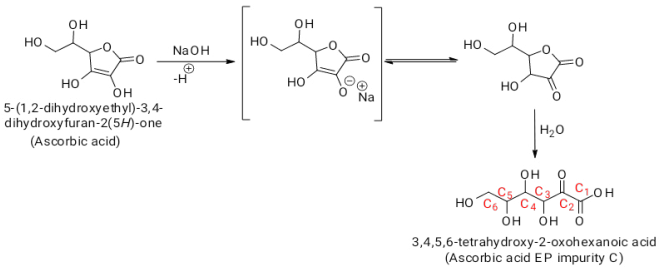

Fig. 10: The proposed mechanism for the conversion of 5-(1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)-one (Ascorbic acid) to 3,4,5,6-tetrahydroxy-2-oxohexanoic acid (Ascorbic acid EP impurity C).

Photolytic Degradation

In 200 mg of ascorbic acid was exposed to the light of 254 and 365 nm for up to 10 days. The chromatographic studies of the withdrawn samples showed no significant degradation. The API remained stable in these parameters under harsh conditions.

Oxidative Degradation

One gram of ascorbic acid was dissolved in 10 volumes of hydrogen peroxide (30%) and reacted for 4 hours at room temperature. The chromatographic studies of the sample withdrawn showed degradation. The degradant is water soluble in nature. Isolation and identification of the degradation compounds in oxidative conditions are under study.

The degradation profile of ascorbic acid is shown in Table 2. Ascorbic acid, a flavonoid, is an important antioxidant. The degradation study of ascorbic acid was undertaken in this work as per ICH guidelines. The behavior of the degradation process under acidic, alkaline, thermal, photolytic, and oxidative environments was explored. Very encouraging results were found for alkaline degradation, where the main degraded product was revealed to be (3S,4R,5S)-3,4,5,6-tetrahydroxy-2-oxohexanoic acid (Ascorbic acid EP impurity C), which is one of the pharmacopeial impurities

of ascorbic acid. ((3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxo hexanoate) (ascorbic acid EP impurity D) was created by isolating the molecule (impurity C, 95.06 %) and converting it to its ester. When ascorbic acid was degraded, EP impurity C was sure to form since the synthesis of (3S,4R,5S)-methyl 3,4,5,6-tetrahydroxy-2-oxohexanoate) was possible from the isolated compound. Based on its analytical and spectral data (^{1}H-NMR, DMR, MS), both impurities were identified. The API also deteriorated in acidic, thermal, oxidative, and photolytic environments. In oxidative conditions, when the API was unmasked hydrogen peroxide degradation was noticed. The degraded products are highly water soluble. Isolation and characterization of the degraded products are under process. No notable modifications were observed in acidic, thermal, or photolytic conditions. Consequently, we concluded that ascorbic acid is prominently stable in strong acids and high temperatures (up to 175°C), and ultraviolet radiation (254 and 365 nm).

Plausible Mechanism

The proposed mechanism is depicted in the scheme in the below Fig. 10. The hydroxyl group adjacent to the double bonds undergoes keto-enol tautomerization in the presence of a base. Then the ring gets hydrolyzed to give an open chain structure.

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