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Novel Analytical Approach for the Estimation of Oxalic Acid Content in Sodium Ascorbate by Reversed Phase-High Performance Liquid Chromatography

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A simple and novel reverse phase-high performance liquid chromatography (RP-HPLC) method employing Triart C_{18} column has been developed and validated for the estimation of oxalic acid content in sodium ascorbate drug substance and drug product. Forced degradation study of sodium ascorbate was performed at acid, base, oxidative, photolytic and thermal stress conditions. Oxalic acid was found as final degradants. The separation was achieved in isocratic mode using mixture of tetrabutylammonium hydroxide-phosphate buffer (pH 7.0) and acetonitrile in the ratio of 80:20; v/v as mobile phase at a flow rate of 1.0 mL min^{-1} using UV detector. The method was validated as per the ICH guideline (Q2R1) for accurate and precise quantification of oxalic acid content in sodium ascorbate. The obtained limit of quantification of oxalic acid was 0.034%. Mean recovery of oxalic acid was found to be 98.9 ± 8.7%. Presented method could be applied for the estimation of oxalic acid content in sodium ascorbate at pharmaceutical laboratories.

Keywords: Sodium ascorbate, Ascorbic acid, Oxalic acid, Forced degradation, Method validation.

INTRODUCTION

Sodium ascorbate, a mineral form of ascorbic acid (vitamin C), chemically is sodium (2*R*)-2-[(1*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2*H*-furan-3-olate (*m.f.*: $C_6H_7NaO_6$ and *m.w.*: 198.11) [1,2]. Sodium salt use of the vitamin C helps to be easily absorbed, stay longer in the body and lower the acidity levels [3,4]. Sodium ascorbate is the most widely taken as nutritional supplement and is available in a variety of dosage forms including tablets, capsules, in multivitamin/mineral formulation, drink mixes packets and as crystalline powder [2,5,6]. Sodium ascorbate is used to prevent and treat scurvy. Scurvy is characterized by spots on and bleeding under the skin, spongy gums, corkscrew hair growth, bone pain, bleeding gums and poor wound healing [7-9]. Sodium ascorbate is an essential nutrient involved in the repair of tissue and enzymatic production of neurotransmitters. It is required for the functioning of several enzymes and important for immune system function as an antioxidant [10].

Literature survey reveals that the supplement of sodium ascorbate reduces the duration and severity of common cold, even, in upper respiratory tract infections [11,12]. In a current Covid-19 pandemic situation, sodium ascorbate plays an important role to increase the immune system of humans and is used widely all over the world [13,14]. The normal range of dietary intake supplementation of sodium ascorbate lowers the risk for developing cancer cells. Once cancer is diagnosed, large amounts of sodium ascorbate given intravenously treat the cancer, reduce the side effects of other treatments and improve quality of life [15,16]. Lower vitamin C concentration level is found in people diagnosed with Alzheimer's disease and supplementation in the treatment of rheumatoid arthritis [17].

Recommendations for vitamin C intake by adults have been proposed by various National Health agencies: US and Health Canada; 90 mg per day and a tolerable upper intake level US; 2000 mg per day. Approximately 70-80% of sodium ascorbate is absorbed in a moderate intake of 30-180 mg per day, at above 1000 mg per day adsorption falls to less than 50%. Dehydroascorbic acid (DHA) is absorbed higher rate than ascorbate, the amount of DHA found in plasma and tissues. Sodium ascorbate is a water soluble diet with dietary excess

not absorbed and excess in the blood being rapidly eliminated in urine [2,3,18,19].

The most commonly used some dietary supplement compounds are ASA, sodium ascorbate (SA), calcium ascorbate and potassium ascorbate [3,4]. ASA also converts (reversibly) to DHA and from that compound non-reversibly to 2,3-diketogulonate and then oxalate [19]. These three compounds are also excreted *via* urine. The final oxidative product of ASA is oxalate *i.e.* oxalic acid. Oxalic acid is toxic because of its acidic and chelating properties. The oxalic acid can combine with calcium and iron to produce an insoluble solid, calcium oxalate and iron oxalate. There is a longstanding excess of ASA, the body can break down these and eliminate them *via* the kidney or colon which increases the risk of kidney stones. About 80% of kidney stones are entire of calcium oxalate type [20-22]. So control of oxalic acid is a major concern in the ASA and sodium ascorbate.

The literature survey revealed that several HPLC methods are reported for the determination of oxalic acid in ASA and sodium ascorbate *e.g.* Kim & Kim [23] reported HPLC-UV method for simultaneous determination of ASA and DHA in human plasma. Linda *et al.* [24] reported the identification of oxidation products of L-ASA by HPLC. Clark & Frank [25] reported the HPLC-ECD method for the quantification of ASA in human plasma [25], whereas Zbynek *et al.* [26] determined ASA using HPLC coupled with the electrochemical method. Yang *et al.* [27] reported the determination of oxalic acid, oxamic acid, and oxamide in a drug substance by ion exclusion chromatography. Furthermore, all these methods were found to have limited access for determination of oxalic acid in the ASA and sodium ascorbate [23-27]. During the force-degradation studies, oxalic acid has been generated as degradation impurity. Additional two USP impurities *viz.*, impurity-C and impurity-D are the process related impurities. The route of degradation pathway of sodium ascorbate showed in Fig. 1. To the best of our knowledge, no method was reported earlier for the determination of oxalic acid in the drug substance sodium ascorbate using HPLC for the regular analysis and stability studies in the quality control laboratories.

The core objective of this research work is to develop a simple stability indicating RP-HPLC method for the determination of oxalic acid in sodium ascorbate. The study of forced degradation was carried out according to the conditions recommended by the ICH *i.e.* acid, base, oxidative, thermal and photolytic stressed conditions to prove the stability indicating ability of the method. The mixture of the degraded sample and its related impurities were used to optimize the method. The optimized method was validated as per regulatory guidelines [28,29].

EXPERIMENTAL

Oxalic acid (99.9%) was procured from Merck (India). The HPLC grade solvents and acetonitrile were also procured from Merck (India). Other AR grade reagents *viz.* tetrabutylammonium hydroxide (TBA-OH) ~ (40% in water), potassium dihydrogen orthophosphate, orthophosphoric acid, sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from Sd. Fine Chemicals Ltd. (India). HPLC grade water was obtained from Millipore system (Millipore Inc., USA) and used throughout the study. Sodium ascorbate (99.9%) was obtained as gratis sample.

HPLC conditions: The Agilent 1200 series HPLC system (Agilent Technologies, Germany) with a photodiode array detector (PDA) was used. The Triart C_{18} (150 × 4.6 mm, 5 µm, YMC Technologies, USA) column was used for the method development and method validation studies. Buffer solution is the mixture of 10 mL of tetrabutylammonium hydroxide solution ~ (40% in water) and 0.01 M potassium dihydrogen orthophosphate solution, adjusted the pH 7.00 ± 0.05 using dilute orthophosphoric acid solution. Filtered this solution through 0.45 µm membrane filter (Millipore PVDF) and degassed in ultrasonic bath. The buffer solution and acetonitrile in the ratio of 80:20; v/v was used as mobile phase. The flow rate, auto-sampler temperature and injection volumes were used 1.0 mL min^{-1}, 10 ºC and 10 µL, respectively. The column oven was set at 30 ºC. The analysis was carried out under isocratic mode. Total runtime for the analysis was 10 min. Chromatographic data was acquired at 220 nm. Data acquisition and

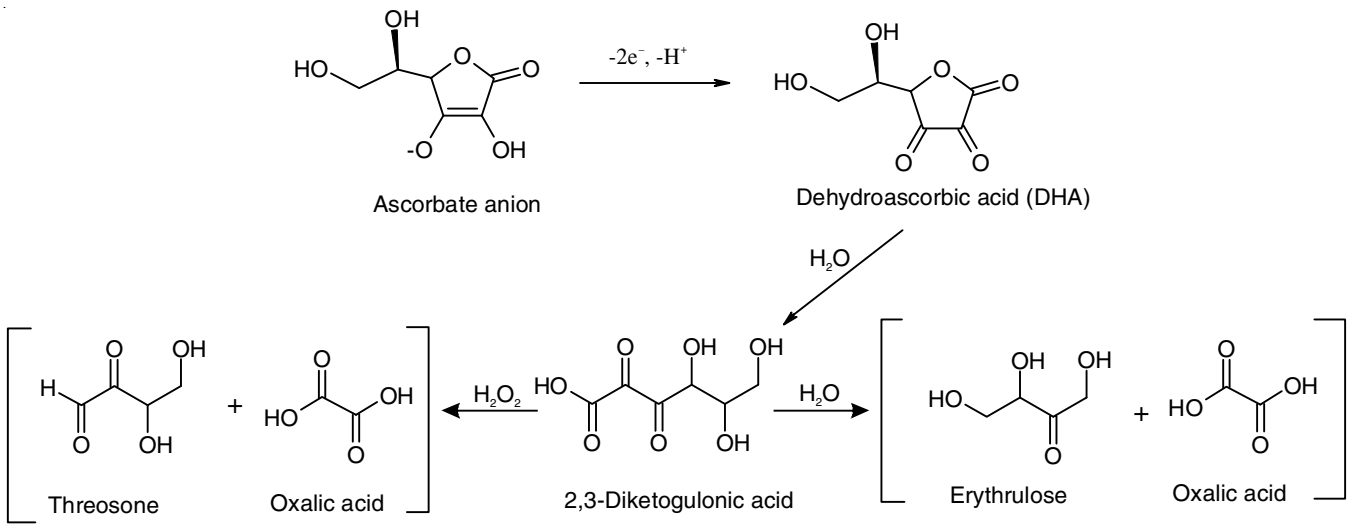

Fig. 1. Degradation pathway of ascorbate anion

processing was performed using Chromeleon 6.8 chromatographic software. For the analysis of forced degradation samples, the PDA detector was used in the scan mode from 200 nm to 400 nm. The peak homogeneity was expressed in terms of peak purity values.

Preparation of analytical solutions: A degassed buffer solution containing mixture of 10 mL of tetrabutylammonium hydroxide solution (40% in water) and 0.01 M potassium dihydrogen orthophosphate solution, adjusted the pH 5.50 ± 0.05 using dilute orthophosphoric acid solution. The buffer solution and acetonitrile in the ratio of 80:20; v/v was used as diluent. The sample solution was prepared at concentration of 1000 μg mL^{-1} in diluent. The standard solution of oxalic acid (5 μg mL^{-1}) prepared in diluent (0.5% with respect to test concentration). System suitability solution was prepared concentration of oxalic acid and sodium ascorbate at 5 μg mL^{-1}, respectively, the system suitability solution was used to verify the performance of the column and instrument. The standard solution was used for quantification of oxalic acid.

Method validation

Specificity: The specificity of developed HPLC method was demonstrated by analyzing the diluent, test sample, test sample spiked with oxalic acid at their specification level and impurity-C and impurity-D individually. Their spectral peak purity data was recorded.

Forced degradation studies: The forced degradation studies were performed to demonstrate the stability-indicating properties of the established method. The stressed conditions engaged for degradation studies as per ICH recommendation include acid hydrolysis, base hydrolysis, oxidative, thermal and photolytic stressed conditions. For acid and base hydrolysis, the sample was treated by using 1 mL of 0.1 N HCl solution for 30 min and 1 mL of 0.1 N NaOH solution for 10 min at ambient temperature, respectively and further neutralized. The oxidation stress was done with 0.5 mL of 3% H_2O_2 solution for 15 min at ambient temperature. The thermal stress study was done at 105 ºC for 3 h. The photolytic stress study was performed by exposing the sample of sodium ascorbate at 1.2 million Lux hours [28]. A photodiode array detector was used to verify and ensure the uniformity and purity of oxalic acid peak in all the stressed sample solutions. Mass balance assessment in the degraded samples was performed to confirm that the impurities detected in the stressed samples corresponded to the amounts present before the stress was applied [30].

Precision: For method precision, six individual measurements of sodium ascorbate sample spiked with oxalic acid were analyzed, calculated the % of oxalic acid from each measurement and determined their mean, standard deviation and relative standard deviation. An intermediate precision experiment performed by different analyst, column and instrument in the same laboratory. Obtained results of method precision and intermediate precision were compared.

Linearity: The linearity of the method was performed in the range of LOQ to 150% of the specification limit of oxalic acid content. The graph of peak response *versus* analyte concentration was plotted, their linear regression determined by the least squares method. The slope and intercept values of the calibration curves have been calculated.

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ of oxalic acid were determined from the calibration curve using the following formulae:

$$LOD = \frac{C_d \times Syx}{b}$$

$$LOQ = \frac{C_q \times Syx}{b}$$

where C_d/C_q is the coefficient for LOD/LOQ; Syx is the residual variance due to regression; b is slope. Precision at obtained LOQ concentrations was also determined.

Accuracy: The recovery experiments were conducted to determine the accuracy of the method for the quantification of oxalic acid in sodium ascorbate. The study was carried out in triplicate at four different concentration levels *viz*. LOQ, 50, 100 and 150% of the specification level. Percentage recoveries were calculated by using the following formula:

$$\text{Recovery } (\%) = \frac{\text{Amount recovered}}{\text{Amount added}} \times 100$$

Robustness: Robustness of the method was evaluated by making deliberate changes in flow rate (± 10%), mobile phase organic composition (± 10% absolute), column oven temperature (± 2 ºC) and buffer solution pH (± 0.2). At each condition determined the resolution between oxalic acid and sodium ascorbate was determined.

All the other mobile phase components were held constant as described above.

Stability of analytical solutions and mobile phase: The solution stability of system suitability solution, sodium ascorbate sample solution and oxalic acid spiked sample solution were carried in auto-sampler at 10 ºC for 24 h. The stability of the mobile phase was confirmed by quantifying freshly prepared sample solutions in accordance with the freshly prepared system suitability solution for 24 h period. During the study period, the prepared mobile phase was kept unvarying. The content of oxalic acid was calculated against the initial value for the study period during mobile phase and solution stability experiments.

RESULTS AND DISCUSSION

HPLC method development: The specifications are one part of an overall control strategy of the drug substance and drug product designed to ensure product quality and consistency. To meet regulatory requirements for establishing specifications (ICH Q6) and qualitative impurities (ICH Q3A (R2)).

The present study aims to develop an effective and useful chromatographic method, which could be capable of eluting and resolving sodium ascorbate, oxalic acid, their degradation products and related impurities within the short run time. Oxalic acid and sodium ascorbate solutions were prepared in diluent at 10 μg mL^{-1} scanned using UV-visible spectrophotometer and showed the UV absorbance at lower wavelength, maxima at around 220 nm and 267 nm, respectively. Therefore,

a wavelength of 220 nm was chosen for detection during the development of this method.

The oxalic acid is the potential impurity and present in the bulk samples, which was produced in the degradation of sodium ascorbate and its retention is early due to polar nature. The core objective of this chromatographic method was to achieve the better peak shape of sodium ascorbate, retain the sodium ascorbate and the separation of closely eluting peaks. Method development trials were initiated in gradient mode. The pK_a values of ASA are 4.2 and 11.6, for oxalic acid are 1.2 and 4.2, indicated that ASA is weakly basic while oxalic acid strong dicarboxylic acid. Method development attempts were initiated at the neutral pH mobile phase. Use of 0.05 M ammonium acetate in water, adjusted pH 7.0 with ammonia solution was selected as buffer for mobile phase-A and 100% acetonitrile for mobile phase-B at slow gradient. At pH 7.0 with volatile buffer, sodium ascorbate and oxalic acid peaks were eluted early with no adequate resolution.

In next experiment, 0.05 M potassium dihydrogen orthophosphate (pH 3.5 with dilute orthophosphoric acid solution) as mobile phase-A and 100% acetonitrile as mobile phase-B in the ratio of 90:10 v/v were selected. The early retention of sodium ascorbate with peak asymmetry more than 3.5 was observed. Tried the same experiment by changing the buffer pH 8.0 and 8.5,no improvement was observed in retention and asymmetry. Also sample solution found unstable at pH 3.5, 8.0 and 8.5. To overcome these problems, 0.01 M potassium dihydrogen orthophosphate solution adjusted pH 7.0 with KOH solution was used as a buffer. Water was used as diluent for sample preparation. Improvement in the asymmetry of sodium ascorbate was observed with same retention. Hence, we changed our strategy of method development, by using ion pair reagents as buffer to retain the analytes peaks. During the method development trials with adding 0.1% of 1-butane sulfonic acid as a ion pair in the mobile phase of buffer pH 7.0, sodium ascorbate and oxalic acid were well retained. Similar observations were observed with 1-pentane sulfonic acid and 1-octane sulfonic acid. However, the resolution between sodium ascorbate and oxalic acid was less than 2.0. Further experimentation with 10% tetrabutylammonium hydroxide in water as a buffer resulted in the reasonable retention and resolution with noisy baseline. To overcome noise baseline issue, switched to isocratic mode from gradient. Improvement in the asymmetry and retention of the oxalic acid and sodium ascorbate were using 20% tetrabutylammonium hydroxide in water as a buffer. Mixture of 10 mL of tetrabutylammonium hydroxide (TBA-OH) and 0.01 M potassium dihydrogen orthophosphate with acetonitrile was optimized for retention of analyte. Development trials for the quantity optimization of 40% tetrabutylammonium hydroxide solution in mobile phase are summarized in Table-1. Different make columns of same stationary phase were tested in order to verify the performance of the optimized method. Data is tabulated in Table-2 and representative chromatograms are shown in Fig. 2a. USP impurities *viz*., impurity-C and impurity-D are the process related impurities which were well resolved from both sodium ascorbate and oxalic acid (Fig. 2b).

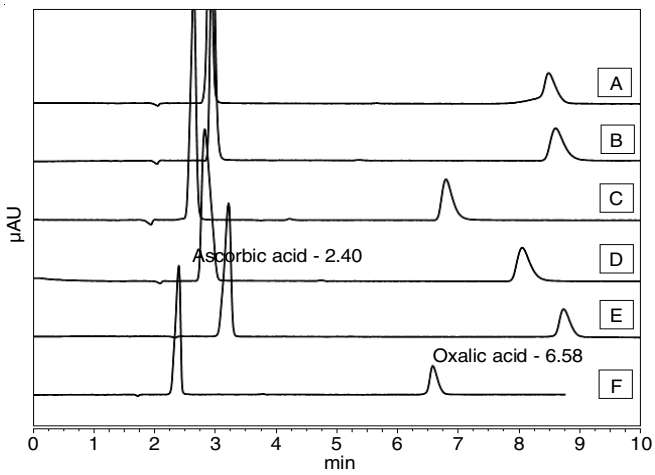

Fig. 2a. Typical method development chromatograms using different columns [A: Zorbax SB C18; B: Purospher STAR RP-18e; C: Inertsil ODS-3V; D: Kromasil C18; E: Hypersil ODS and optimized method on F: Triart C18 all column dimension is (150 × 4.6 mm, 5 µm)]

Optimum peak shape and resolution between the closely eluting peaks was achieved on a YMC Triart C_{18} column (150 × 4.6 mm and 5.0 µm particle size, YMC Technologies, USA) and equivalent column Inertsil ODS-3V (150 × 4.6 mm, 5 µm)

TABLE-1
METHOD DEVELOPMENT TRIALS AND OBSERVATIONS FOR CHANGING IN VOLUME OF TETRABUTYLAMMONIUM HYDROXIDE ~ (40% IN WATER) SOLUTION IN MOBILE PHASE

Volume of 40% of TBA-OH (mL)*	RT of sodium ascorbate (min)	RT of oxalic acid (min)	RRT w.r.t. sodium ascorbate	Resolution (R_s)	USP tailing factor (T) (oxalic acid)	Observations
2	1.52	3.32	2.18	9.1	0.98	Sodium ascorbate peak that is eluted near the column dead volume.
5	2.04	4.38	2.15	11.7	1.01	Poor resolution observed.
8	2.21	5.56	2.52	16.3	1.02	Poor resolution observed.
10	2.35	6.45	2.75	24.7	1.03	Present work
12	2.40	7.08	2.95	22.8	1.04	More tailing factor of oxalic acid was observed.
15	2.57	8.27	3.22	26.5	1.07	Retained the oxalic acid peak and tailing was observed.
20	3.18	9.36	2.94	27.4	1.14	Tailing observed for both analyte.

*Diffrent volume (mL) of tetrabutylammonium hydroxide ~ 40% in water (TBA-OH) with 0.01 M potassium dihydrogen orthophosphate solution, adjusted the pH 7.00 ± 0.05 using orthophosphoric acid, the buffer solution and acetonitrile in the ratio of 80:20; v/v was used as mobile phase and using same method parameters.

TABLE-2
METHOD DEVELOPMENT TRIALS AND OBSERVATIONS

Trials	Column (dimension)	Analyte	Retention time (min)	Relative retention time	Resolution (R_s)	USP tailing factor (T)	Observations
1	Zorbax SB C18 (250 × 4.6 mm 5.0 µ)	Sodium ascorbate	2.91	1	N.A.	0.94	Fronting observed for both analyte.
		Oxalic acid	8.49	2.92	22.47	0.82	
2	Purospher STAR RP-18e (250 × 4.6 mm 5.0 µ)	Sodium ascorbate	2.95	1	N.A.	1.15	More tailing factor of oxalic acid was observed.
		Oxalic acid	8.60	2.92	20.13	1.32	
3	Inertsil ODS-3V (250 × 4.6 mm 5.0 µ)	Sodium ascorbate	2.66	1	N.A.	0.98	Tailing factors of both analyte were less than 1.2
		Oxalic acid	6.81	2.56	15.02	1.18	
4	Kromasil C18 (250 × 4.6 mm 5.0 µ)	Sodium ascorbate	2.82	1	N.A.	1.27	Tailing observed for both analyte.
		Oxalic acid	8.04	2.85	14.95	1.33	
5	Hypersil ODS (250 × 4.6 mm 5.0 µ)	Sodium ascorbate	3.22	1	N.A.	0.76	Sodium ascorbate peak fronting and oxalic acid peak tailing was observed.
		Oxalic acid	8.73	2.71	20.42	1.24	
6	Triart C18 (250 × 4.6 mm 5.0 µ)	Sodium ascorbate	2.40	1	N.A.	0.96	Optimized method.
		Oxalic acid	6.58	2.75	21.3	1.12	

Mobile phase: The mixture of 10 mL of tetrabutylammonium hydroxide ~ 40% in water (TBA-OH) with 0.01 M potassium dihydrogen orthophosphate solution, adjusted the pH 7.00 ± 0.05 using dilute orthophosphoric acid, the buffer solution and acetonitrile in the ratio of 80:20; v/v. The analysis was carried out under isocratic conditions. Endeavors completely different make columns of the same stationary phase were tried.

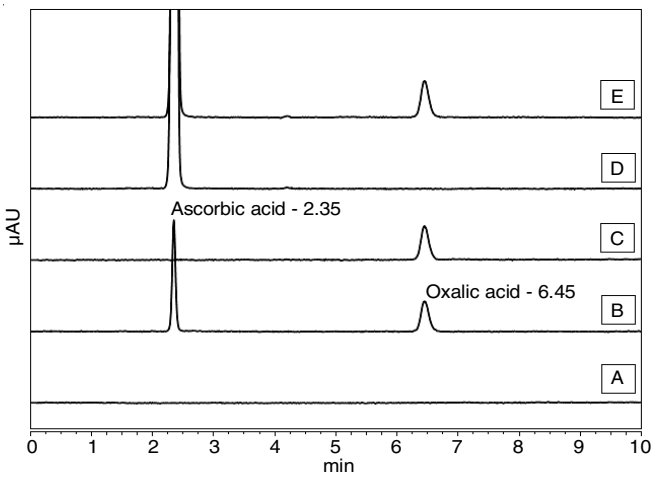

Fig. 2b. Typical RP-LC chromatograms of; (A) Blank solution, (B) System suitability solution, (C) Standard solution, (D) Sodium ascorbate test and (E) Spiked with oxalic acid solution

column using buffer solution of 0.01 M potassium dihydrogen orthophosphate solution with 10 mL of tetrabutylammonium hydroxide (TBA-OH). Adjusted buffer pH at 7.00 ± 0.05 using dilute orthophosphoric acid solution and filtered through 0.45 µm membrane filter (Millipore PVDF) and degassed in ultrasonic bath. The buffer and acetonitrile in the ratio of 80:20; v/v was used as mobile phase. The flow rate, auto-sampler temperature and injection volumes were used 1.0 mL min^{-1}, 10 ºC and 10 µL, respectively. The column oven temperature is 30 ºC. The analysis was carried out under isocratic conditions. Runtime for the analysis was 10 min and chromatographic data was acquired at 220 nm, the retention time of sodium ascorbate and oxalic acid at the optimized method was appropriate and the tailing factor of sodium ascorbate and oxalic acid were found to be less than 1.20. In the optimized conditions, it was observed that sodium ascorbate and oxalic acid were well separated with a greater resolution than 10.0 (Fig. 2b).

The typical retention times of sodium ascorbate and oxalic acid were about 2.3 and 6.6 min, respectively. The system suitability results are shown in Table-3 and the developed HPLC method was found to be specific for sodium ascorbate, its known impurities and degradation impurities (Fig. 3a-b). The developed method was also applied to pharmaceutical drug product, which describes the separation of impurities in formulations (Fig. 4). The peaks were identified by injection

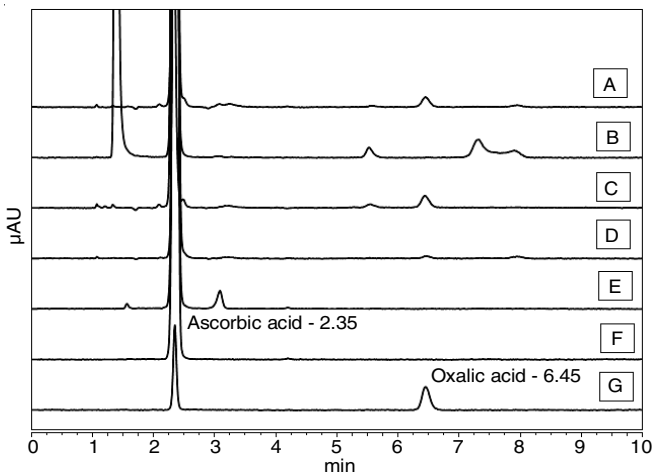

Fig. 3a. Typical RP-LC chromatograms of (A) Acid degradation, (B) peroxide degradation, (C) Base degradation, (D) Thermal degradation, (E) Photo degradation, (F) Sodium ascorbate test, (G) System suitability solution

TABLE-3
CHROMATOGRAPHIC PERFORMANCE DATA

Parameter/Analyte	Retention time (min)	Relative retention time	Resolution (R_s)	USP tailing factor (T)	Number of theoretical plates
Sodium ascorbate	2.35	1.00	N.A.	1.03	7951
Oxalic acid	6.45	2.75	24.7	1.03	12936

TABLE-4
FORCED DEGRADATION RESULTS

Stress condition	Duration	Oxalic acid content (%, w/w)	Degradation observed (%, w/w)	Mass balance (%, w/w)	Remark
Acid hydrolysis	1 mL of 0.1N HCl, at RT for 30 min	0.8	9	99.4	Significantly formed oxalic acid
Base hydrolysis	1 mL of 0.1N NaOH, at RT for 30 min	1.0	12	99.6	Significantly formed oxalic acid
Oxidation	3% H_2O_2 at RT for 15 min	0.2	18	99.3	Significantly degradation formed
Thermal	105 °C for 3h	0.3	6.1	99.5	Significantly formed oxalic acid
Photolytic	1.2 Lux hours	0.1	8.6	99.6	Significantly degradation formed

Mass balance = % sodium ascorbate + % oxalic acid content + % sum of all degradents, RT = Room temperature.

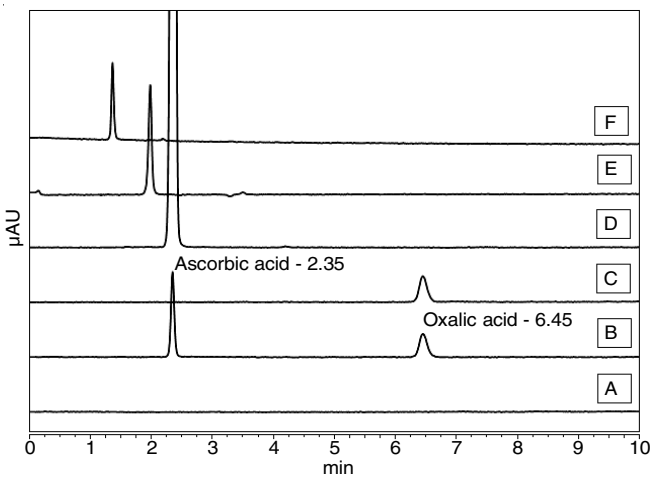

Fig. 3b. Typical RP-LC chromatograms of (A) Blank solution, (B) System suitability solution, (C) Standard solution, (D) Sodium ascorbate test, (E) Impurity-C solution and (F) Impurity-D solution

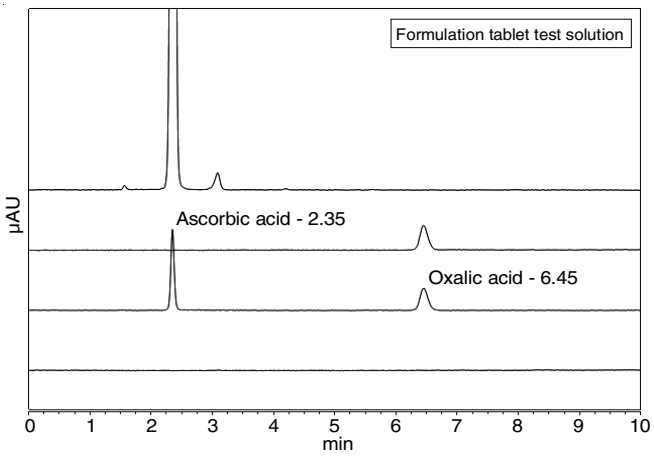

Fig. 4. Typical RP-LC chromatograms of formulation tablet test solution

and compared with the retention times of the individual compounds and by studying the absorption spectra using a PDA detector. An unknown impurity has eluted close to the oxalic acid was well separated under the optimized conditions.

Forced degradation studies: In acid hydrolysis, significantly oxalic acid was formed with total 9% w/w degradation. In base hydrolysis significantly oxalic acid was formed with total 12% w/w of degradation. The oxidation stress studies 18% w/w degradation was observed with oxalic acid and unknown impurities. Photolytic stress studies was performed for 5 h and thermal stress was done at 105 ºC for 3 h degradation. The peak purity test results obtained from PDA confirmed that the oxalic acid peak is homogeneous and pure in all the stressed samples analyzed. The mass balance is a process of adding together the oxalic acid content value and the levels of degradation products to see how closely these add up to 100% of the initial value with due consideration of the margin of analytical error [31]. The mass balance of stressed samples was close to 99.6% (Table-4). The percentage of oxalic acid was unaffected in presence of known and unknown impurities (Fig. 3a-b), thus, confirmed the stability-indicating of the developed method.

The obtained % RSD of oxalic acid content for precision study was 0.40% while for intermediate precision study was within 0.79%. The cumulative% RSD (n = 12) for oxalic acid content was 0.63%. The individual values were found well within the range of confidence interval of the average confirming good precision of the method.

The LOD and LOQ of oxalic acid were 0.011% w/w and 0.034% w/w respectively (Table-5). The peak area precision at LOQ level was below 10%. The percentage recovery of oxalic acid ranged from 99.3 to 99.7%. HPLC chromatogram of oxalic acid spiked in sodium ascorbate bulk sample is provided in Fig. 2b.

TABLE-5
VALIDATION DATA OF OXALIC ACID CONTENT IN SODIUM ASCORBATE

Compound	Oxalic acid content (%, w/w)
Precision	
% RSD in method precision (n = 6)	0.40
% RSD in Intermediate precision (n = 6)	0.79
Mean $\bar{x}$ (n = 12)	0.505
± S.D. (n = 12)	0.003
Overall% RSD (n = 12)	0.63
Linearity	
Correlation coefficient (r)	0.9998
Slope	278.0956
Intercept	-0.9819
Limit of detection (%, w/w)	0.011
Limit of quantitation (%, w/w)	0.034
Accuracy mean accuracy (%) (n = 3)	99.6

In all the deliberate varied chromatographic conditions carried out as described above (flow rate, organic ratio, column temperature and pH of buffer), the tailing factor of oxalic acid was less than 1.20 and the resolution between sodium ascorbate and oxalic acid peaks was greater than 20. No significance change in resolution and tailing factor under all robustness conditions illustrating the robustness of the method. The robustness data is shown in Table-6.

TABLE-6
ROBUSTNESS DATA OF OXALIC ACID CONTENT IN SODIUM ASCORBATE

Parameter/Analyte	Retention time (min)	Resolution (R_s)	USP tailing factor (T)	Retention time (min)	Resolution (R_s)	USP tailing factor (T)
Column oven temp.		(+2°C)			(-2°C)	
Sodium ascorbate	2.36	-	1.02	2.32	-	1.04
Oxalic acid	6.48	24.6	1.05	6.42	24.1	1.03
Organic ratio (acetonitrile)		(+10%)			(-10%)	
Sodium ascorbate	2.32	-	1.03	2.43	-	1.02
Oxalic acid	6.38	23.8	1.02	6.74	23.9	1.06
pH		(+pH 0.2)			(-pH 0.2)	
Sodium ascorbate	2.33	-	1.01	2.37	-	1.02
Oxalic acid	6.46	24.3	1.03	6.49	23.5	1.04
Flow		(+10%)			(-10%)	
Sodium ascorbate	2.29	-	1.01	2.41	-	1.03
Oxalic acid	6.40	24.7	1.02	6.53	23.8	1.06

The% RSD of oxalic acid content during solution stability and mobile phase stability experiments were within 0.5%. The accuracy of the oxalic acid content against the initial value is between 99.4% and 99.5%. The solution stability and mobile phase stability experimental data confirms that sample solutions and mobile phase were stable up to 24 h.

Application: Three commercial batches of sodium ascorbate drug substance and drug product were analyzed using validated method. The analysis results of the commercial drug substance are shown in Table-7. It indicates that the method is specific and selective for the qualitative and quantitative determination of oxalic acid content in the formulation and bulk drug samples.

TABLE-7
DATA OF DRUG PRODUCT AND
BULK DRUG SAMPLES ANALYSIS

Sample	Oxalic acid content (%, w/w)
Drug product analysis results (% w/w)	
Formulation-1	Not detected
Formulation-2	Not detected
Formulation-2	Not detected
Bulk drug analysis results (% w/w)	
Test substance batch-1	Not detected
Test substance batch-2	Not detected
Test substance batch-3	Not detected

Conclusion

In conclusion, a rapid, simple, specific and accurate RP-HPLC method has been developed for the quantification of oxalic acid content in sodium ascorbate that separates oxalic acid and potential impurities (impurity-C and impurity-D) generated during the chemical synthesis of ASA. The developed method was validated to ensure the compliance in accordance with the regulatory guidelines. The method was found to be sensitive, accurate, precise, robust and useful for its intended use. The method could not be useful only for routine evaluation of the quality of sodium ascorbate in drug substance manufacturing unit but also for the detection of impurities in drug product.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. W.N. Haworth and E.L. Hirst, *Chem. Ind.*, **52**, 645 (1933); https://doi.org/10.1002/jctb.5000523107
2. A.N. Hiatt, M.G. Ferruzzi, L.S. Taylor and L.J. Mauer, *Int. J. Food Prop.*, **14**, 1330 (2011); https://doi.org/10.1080/10942911003650338
3. H. Sohi, Y. Sultana and R.K. Khar, *Drug Dev. Ind. Pharm.*, **30**, 429 (2004); https://doi.org/10.1081/DDC-120037477
4. X. Yin, K. Chen, H. Cheng, X. Chen, S. Feng, Y. Song and L. Liang, *Antioxidants*, **11**, 153 (2022); https://doi.org/10.3390/antiox11010153
5. S.J. Devaki, R.L.Raveendran and A.H. Hamza, Vitamin C: Sources, Functions, Sensing and Analysis, IntechOpen (2017); https://doi.org/10.5772/intechopen.70162
6. K. Pathy, *SF J. Chem. Res.*, **2**, 1 (2018); https://doi.org/10.32474/SCSOAJ.2018.01.000114
7. B. Marriott, D. Birt, V. Stallings and A. Yates, Vitamin C, Present Knowledge in Nutrition, Academic Press London, pp. 155-170 (2020).
8. M.W. Davey, M.V. Montagu, D. Inze, M. Sanmartin, N. Smirnoff, I.J. Benzie, A. Kanellis, J.J. Strain, D. Favell and J. Fletcher, *J. Sci. Food Agric.*, **80**, 825 (2000); https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6
9. E. Magiorkinis, A. Beloukas and A. Diamantis, *Eur. J. Intern. Med.*, **22**, 147 (2011); https://doi.org/10.1016/j.ejim.2010.10.006
10. R.L. Schleicher, M.D. Carroll, E.S. Ford and D.A. Lacher, *Am. J. Clin. Nutr.*, **90**, 1252 (2009); https://doi.org/10.3945/ajcn.2008.27016
11. H. Hemila and E. Chalker, *Cochrane Database Syst. Rev.*, **2013**, CD000980 (2013); https://doi.org/10.1002/14651858.CD000980.pub4
12. P. Vorilhon, B. Arpajou, H. Vaillant Roussel, É. Merlin, B. Pereira and A. Cabaillot, *Eur. J. Clin. Pharmacol.*, **75**, 303 (2019); https://doi.org/10.1007/s00228-018-2601-7
13. R.Z. Cheng, *Med. Drug Discovery*, **5**, 100028 (2020); https://doi.org/10.1016/j.medidd.2020.100028
14. G.P. Milani, M. Macchi and A. Guz-Mark, *Nutrients*, **13**, 1172 (2021); https://doi.org/10.3390/nu13041172
15. B.V. Sunil Kumar, S. Singh and R. Verma, *Crit. Rev. Food Sci. Nutr.*, **57**, 2623 (2017); https://doi.org/10.1080/10408398.2015.1064086
16. D. Mastrangelo, L. Massai, G. Fioritoni and F.L. Coco, Eds.: A.H. Hamza, Vitamin C Against Cancer, Intech Open (2017); https://doi.org/10.5772/intechopen.68746

17. F.E. Harrison, *J. Alzheimers Dis.*, **29**, 711 (2012); https://doi.org/10.3233/JAD-2012-111853
18. K. Yoshii, K. Hosomi, K. Sawane and J. Kunisawa, *Front. Nutr.*, **6**, 48 (2019); https://doi.org/10.3389/fnut.2019.00048
19. C.L. Linster and E. Van Schaftingen, *FEBS J.*, **274**, 1 (2007); https://doi.org/10.1111/j.1742-4658.2006.05607.x
20. J.S. Goodwin and M.R. Tangum, *Arch. Intern. Med.*, **158**, 2187 (1998); https://doi.org/10.1001/archinte.158.20.2187
21. K.A. Naidu, *Nutr. J.*, **2**, 7 (2003); https://doi.org/10.1186/1475-2891-2-7
22. L.D.K. Thomas, C.G. Elinder, H.G. Tiselius, A. Wolk and A. Akesson, *JAMA Intern. Med.*, **173**, 386 (2013); https://doi.org/10.1001/jamainternmed.2013.2296
23. Y. Kim and M.G. Kim, *TCP*, **24**, 37 (2016); https://doi.org/10.12793/tcp.2016.24.1.37
24. L. Lloyd, Analysis of the Purity and Storage Stability of L-Ascorbic Acid Powders. Application Note, Agilent Technologies, Inc. (2011); https://www.agilent.com/cs/library/applications/5990-8162EN.pdf
25. Z.D. Clark and E.L. Frank, *Pract. Lab. Med.*, **6**, 25 (2016); https://doi.org/10.1016/j.plabm.2016.09.001
26. Z. Gazdik, O. Zitka, J. Petrlova, V. Adam, J. Zehnalek, A. Horna, V. Reznicek, M. Beklova and R. Kizek, *Sensors (Basel)*, **8**, 7097 (2008); https://doi.org/10.3390/s8117097
27. L. Yang, L. Liu, B.A. Olsen and M.A. Nussbaum, *J. Pharm. Biomed. Anal.*, **22**, 487 (2000); https://doi.org/10.1016/S0731-7085(00)00230-2
28. I.C.H. Guideline, Q2(R1) Validation of Analytical Procedures: Text and Methodologies, International Conference on Harmonization, IFPMA, Geneva (2005).
29. The United States Pharmacopeia, Validation of Compendial Methods, Ed.: 35 (2017).
30. R. LoBrutto, A. Jones, Y.V. Kazakevich and H.M. McNair, *J. Chromatogr. A*, **913**, 173 (2001); https://doi.org/10.1016/S0021-9673(00)01012-8
31. M. Bakshi and S. Singh, *J. Pharm. Biomed. Anal.*, **28**, 1011 (2001); https://doi.org/10.1016/S0731-7085(02)00047-X