Ind. J. Pharm. Edu. Res., 2026; 60(2):852-860.
https://www.ijper.org

Original Article

Development and Validation of UPLC-PDA and UV Methods for the Quantitative Estimation of Ascorbic Acid (ASAC) in Pharmaceutical Formulations

Wasim Ahmad[1,*], Nuzhat Banu[1], Ayaz Ahmad[1], Yousif Amin Hassan[1], Manal Surour[1], Rawan Rashad Aleid[1], Munfis Samir Patel[2], Sharell Lewis[3], Kamran Ashraf[4]

[1]Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam, SAUDI ARABIA.
[2]Department of Foundation year, Mohammed Al-Mana College for Medical Sciences, Dammam, SAUDI ARABIA.
[3]Department of Nursing, Mohammed Al-Mana College for Medical Sciences, Dammam, SAUDI ARABIA.
[4]Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Kampus Puncak Alam, Selangor Darul Ehsan, MALAYSIA.

ABSTRACT

Background: Vitamin C, also called Ascorbic Acid (ASAC), is a potent antioxidant that has a significant role in fighting infections and helps in healing wounds. **Aim:** Quantitative estimation of ASAC in formulations is of significance in quality control. A simple, sensitive, highly efficient and reliable Ultra-Performance Liquid Chromatography (UPLC) analytical method was suggested for evaluating ASAC in various pharmaceutical products (chewable tablet, effervescent tablets, and capsules). **Materials and Methods:** The UPLC method was developed on a C8 column, and the column oven temperature was adjusted to 35±5ºC using Empower software. The mobile phase used was water, methanol, and 0.1% formic acid (16:80:04, v/v/v). 1 µL volume was injected on isocratic elution with a flow rate of 0.3 mL/min, and 2 min run time for the separation of ASAC. The peak absorbance of ASAC was noted at 244.3 nm. **Results:** The linearity of the ASAC calibration curve in the range of 25-125 ng/mL and 1-100 µg/mL for the developed UPLC and UV methods, respectively. The values of the correlation coefficient (R^2) for ASAC were assessed to be 0.9987 and 0.9873 by UPLC and UV methods, respectively. The results obtained showed a strong correlation between ASAC concentration and UPLC and UV response. **Conclusion:** The proposed suggested method demonstrated linearity, precision, accuracy, specificity, and robustness, for the quantitative assessment of pharmaceutical formulations consisting of ASAC within a short period of time.

Keywords: UPLC, UV spectrophotometer, TLC, Vitamin C, Marketed formulation.

Correspondence
Dr. Wasim Ahmad
Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam-34222, SAUDI ARABIA.
Email: wasimahmadansari@yahoo.com

Received: 18-08-2025;
Revised: 06-10-2025;
Accepted: 24-12-2025.

INTRODUCTION

Vitamin C, also called Ascorbic Acid (ASAC), is a potent antioxidant that has a significant role in fighting infections and helps in healing wounds. It assists in making collagen, a protein found in connective tissue that constitutes various systems in the body like bones, blood, cartilage, nervous and immune systems, etc. Collagen provides flexibility to the body and maintains a stable structure.[1-3]

Almost all animals synthesize ASAC, but the human body does not produce it and therefore the only source of it is fresh fruits and vegetables like guava, orange, mango, papaya, cabbage, spinach, and mustard leaves. It increases the absorption of iron, calcium, and folic acid in the body. It strengthens the person's immunity, decreases allergies, forms bile in the urinary tract, and eases steroid secretions.[4-7] ASAC is one of the most important water-soluble vitamins with antioxidant, redox, and pH regulator properties.[8]

ASAC is incorporated into various pharmaceutical and cosmetic formulations as a stabilizer, modifier, antioxidant and enhancer, making its quantification essential in pharmaceuticals, food, plant extracts, and other commercial products. It is one of the most utilized vitamins, either independently or in combination with other vitamins.[8-11]

ASAC was reported to prevent scurvy, a medical ailment that happens due to an insufficient of vitamin C in the body.[12] The drug is listed in the different Pharmacopoeias such as BP, USP, Ph. Eur. and BNF 2021.[13-16] The symptom of the deficiency is noted in men when the total body content of ASAC goes below 300 mg, or the intake is less than 10 mg per day.[17-20]

DOI: 10.5530/ijper.20261506

IUPAC name of ASAC (5R)-5-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one. Tablets, injections, syrups and capsules etc., are the various formulations available on the market. Quantitative assays are conducted to determine ASAC content in various commercial formulations.[21-25]

Currently, drug analysis is a very important area in the pharmaceutical sector. It helps in choosing the dosage forms based on drug content and detecting impurities in pharmaceuticals. It is not only beneficial in pharmaceuticals but also helps in detecting the abused drugs in doping cases. Therefore, quantitatively estimating ASAC in formulations and biological fluids helps in averting adverse effects and promotes proper utilization.[26-29]

Numerous analytical methods have been reported in literature for estimating ASAC acid in different commercial formulations, plant extracts, pharmaceuticals, and food by titrimetric, spectrophotometric, electrochemical, fluorometric, enzymatic, and chromatographic methods.[30-35] Titrimetric methods for analyzing ASAC are simple, cost-effective, economical, and adaptable to routine analyses,[36] Spectrophotometric methods have enhanced detection sensitivity and rapid quantification,[37] High performance liquid chromatography (HPLC) is widely regarded for its precision and reliability in ASAC analysis. It effectively separates ascorbic acid from complex sample matrices, allowing for accurate quantification[38] and Enzymatic Methods are highly selective and can be adapted for various sample types.[39] Among the various analytical techniques described in the literature for the assay of ASAC in pharmaceutical formulations, the most widely used is the chromatographic technique.[40-43]

The methods developed for the analysis of ASAC reported in the literature have limited sensitivity, utilized larger solvent volume, time-consuming, therefore, with the current study; the aim was to develop a simple, robust, cost-effective, precise, along with higher sensitivity, low solvent consumption, and rapid analysis.

Ultra-violet (UV) Spectrophotometry is utilized predominantly to determine ASAC because of its simplicity and ability to absorb UV rays. The method is appropriate for use with ASAC tablets, fruits and vegetables.[44] Thin layer chromatography (TLC) has been utilized as a simple analytical method for the identification of ASAC in fruits and vegetables and pharmaceutical preparations using aluminum silica gel plates.[45,46]

The literature has reported Ultra performance liquid chromatography (UPLC) as one of the new liquid chromatographic techniques that enable reduced solvent consumption and less time for the analysis designed in a way to withstand system backup pressure.[47] This study aimed to develop accurate, fast, precise, sensitive, robust, economical, and specific, UPLC method for the identification and quantification of ASAC in pharmaceutical preparations. The efficacy of the method was assessed by analyzing different pharmaceutical formulations of ASAC like tablets and capsules. In addition, a UV spectrophotometer method was also used for the quantification of ASAC, and TLC was used for its identification. The results of the current method (UPLC) were compared with other documented techniques.

MATERIALS AND METHODS

Chemicals

Standard reference ASAC (Purity: HPLC≥99.0%) was procured from Sigma Aldrich. HPLC grade methanol and water were obtained from Chroma solve (Germany). The source of Formic Acid (FA) was E-Merck (Darmstadt, Germany). The remaining reagents were used of analytical grades. Over-the-counter drugs, two tablets were purchased from a community pharmacy in Dammam and Al Khobar, KSA, whereas another tablet and capsules each one boxes were taken from a community pharmacy in India.

UPLC Instrumentation

The analytical technique development was carried out on Waters Acquity H-Class UPLC Photo Diode Array detector (PDA) (Waters, Milford, MA, USA). ASAC was separated on Acquity UPLC CSH™ C8 column (1.7 µm, 2.1×100 mm) adjusted to 35±5ºC temperature, utilizing Empower software. Water, methanol, and 0.1% formic acid (16:80:04, v/v/v) were employed as mobile phase, using 1 µL volume of injection on isocratic elution and at 0.3 mL/min flow rate and 244.3 nm detection wavelength.

Stock Solutions

A standard stock solution of ASAC with a concentration of 250 µg/mL was made ready in a solvent comprising water and methanol (50:50, v/v). All the samples undergone filtration using membrane filters 0.22 µm syringe.

Preparation of Samples

Over the counter four pharmaceutical formulations, out of which two commercial tablets consisting of ASAC 1000 mg, one tablet consisting of ASAC 500 mg, and one capsule comprising ASAC 500 mg were used. All the commercial formulations were coded as Tablet-1 (ASAC:500 mg), Tablet-2 and Tablet-3 (ASAC:1000 mg), and Capsule-1 (ASAC:500 mg). Ten commercial tablets and capsules of each as mentioned above were taken and average weight was calculated separately for each commercial tablet and capsule. A portion of the Tablet-1, Tablet-2, Tablet-3, and Capsule-1 powder was dissolved in 100 mL of mobile phase comprising water and methanol (50:50, v/v), and sonicated for 30 min for complete dissolution. Further, 1 mL of this solution was diluted with 50 mL of mobile phase comprising water and methanol (50:50, v/v) used earlier for analysis. All the samples underwent filtration with a 0.22 µm filter prior analysis.

Method Validation

Validation of the UPLC method was undergone as per guidelines from ICH[48-51] for the determination of ASAC content, it includes validation parameters: specificity, accuracy, precision, system suitability, robustness, linearity, LOD, and LOQ. The specificity of the UPLC technique was confirmed by matching the retention time and peak apex of the test with that of the standard. The newly developed methods linearity was evaluated by creating a plot of peak areas produced by the injection of ASAC against the concentration used for the construction of the calibration curve. The calibration curve was studied for the regression analysis. The procedure's accuracy was evaluated by employing a recovery study by standard accumulation technique at three varying concentrations of ASAC. The known quantity of ASAC was examined and calculated amounts. This investigation was carried out in triplicate. The system's suitability was assessed to guarantee that the system was working accurately. The system suitability parameters including peak area, Retention time (RT), USP tailing, and K prime (Capacity factor or retention factor) were assessed as per ICH guidelines (n=6).

TLC analysis

TLC analysis of standard ASAC (1 mg/mL) and pharmaceutical formulations (20 mg/mL) samples were conducted on 5×10 cm aluminum plates coated of silica gel 60F (particle size: 5 µm) (Merck, Darmstadt, Germany). The application of the sample was done with the help of a capillary tube. Before starting the chromatography, the TLC chamber (CAMAG, Muttenz,

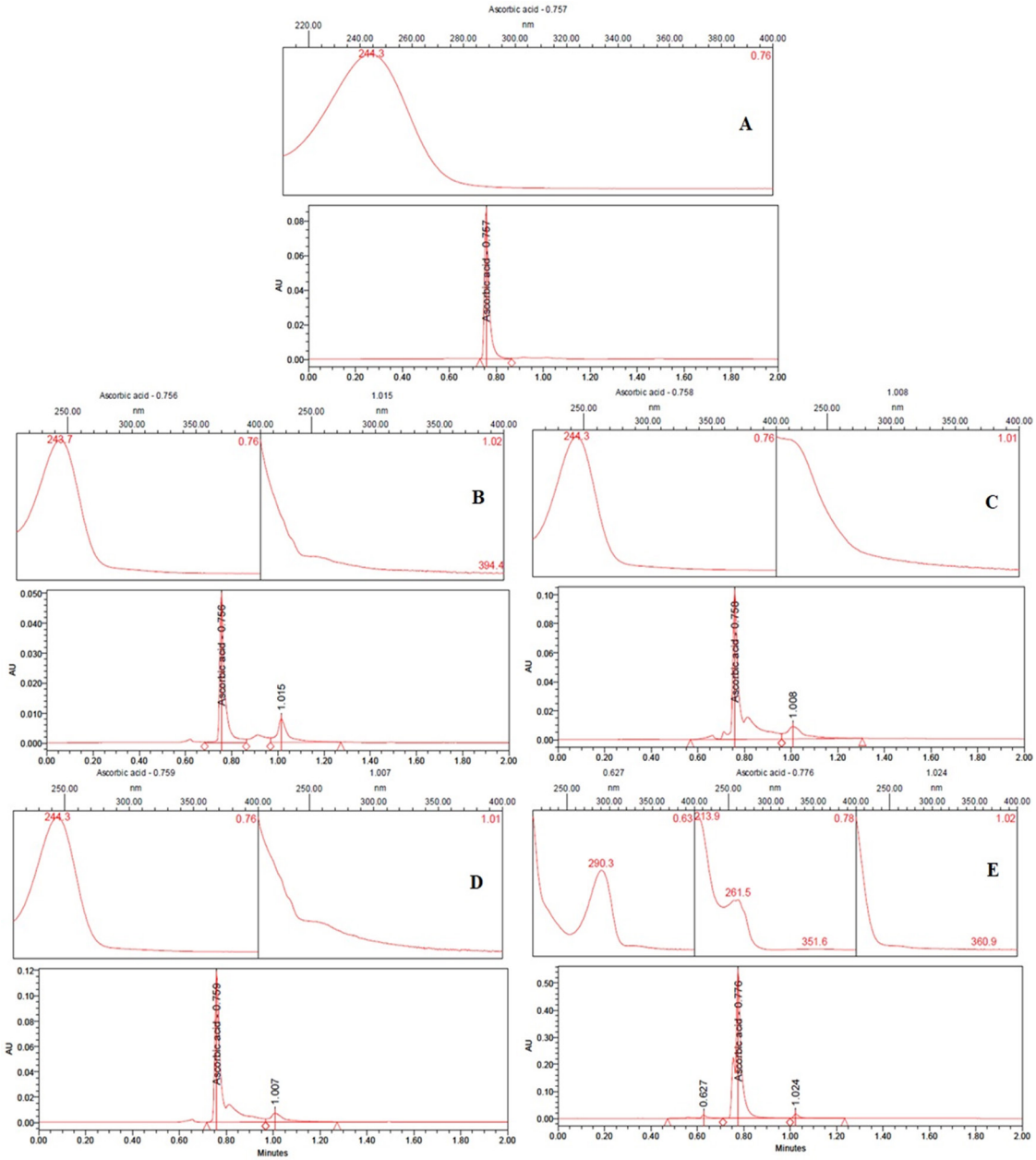

Figure 1: Spectrum index plot: (A) standard ASAC, (B) Tablet-1, (C) Tablet-2, (D) Tablet-3, (E) Capsule-1.

Switzerland) was filled with mobile phase vapors for 15 min to saturate it; 20 mL of the mobile phase was used for the development process. Linear ascending development was done up to 80 mm with solvent system Acetone-toluene-formic acid (6:3:1, v/v/v). Following the development phase, air dryers were utilized to dry the plates.[52] ASAC was spotted at a wavelength of 254 nm using a UV light chamber.

RESULTS

Analytical Method Optimization

Several mixtures of organic or hydro-organic solvents were explored for being used as an eluent system for the setting up of a trustworthy UPLC approach for the identification of ASAC in marketed pharmaceutical formulation samples. The combination of methanol: water, acetonitrile: water, acetonitrile: methanol, methanol: formic acid and acetonitrile: formic acid was among the several solvents that were tested. Several criteria were considered in selecting the best solvent or solvent combination, along with the cost, solvent sensitivity, the duration of the analysis, the chromatographic characteristics, and compatibility with other chemicals. Upon applying the criteria, different solvents: methanol, acetonitrile, water, and formic acid were investigated as the mobile phase individually and in combination. A mixture of Water, methanol, and 0.1% formic acid (16:80;04, v/v/v) showed well separated and intact chromatographic peak of ASAC with a decent value of USP tailing (1.787), and good number of K Prime (6.569), and it also produced consistent R_t (0.757) (Figure 1). Finally, the best separation was achieved on the mixtures of Water, methanol, and 0.1% formic acid (16:80:04, v/v/v) of using the C8 column set up at 35±5°C temperature, using Empower software, using an injection volume 1 µL on isocratic elution with flow rate of 0.3 mL/min and 244.3 nm detection wavelength. Moreover, the maximum absorbance shown by ASAC was at 244.3 nm, when the spectrum index for ASAC was recorded using the PDA mode (Figure 1). Consequently, the assessment of ASAC took place at 244.3 nm within 2 min runtime with a mean retention time of 0.757 (Figure 2).

Method Validation

To assess the several parameters for the analysis of ASAC, the ICH-Q2-R2 criteria was used.[48] The regression of the standard calibration curve helped in assessing the linearity of the procedure. The linearity of the ASAC calibration curve in the range of 25-125 ng/mL and 1-100 µg/mL for the developed UPLC and UV methods, respectively. The values of the correlation coefficient (R^2) for ASAC were assessed to be 0.9987 and 0.9873 by UPLC and UV methods, respectively (Figure 3). The results obtained

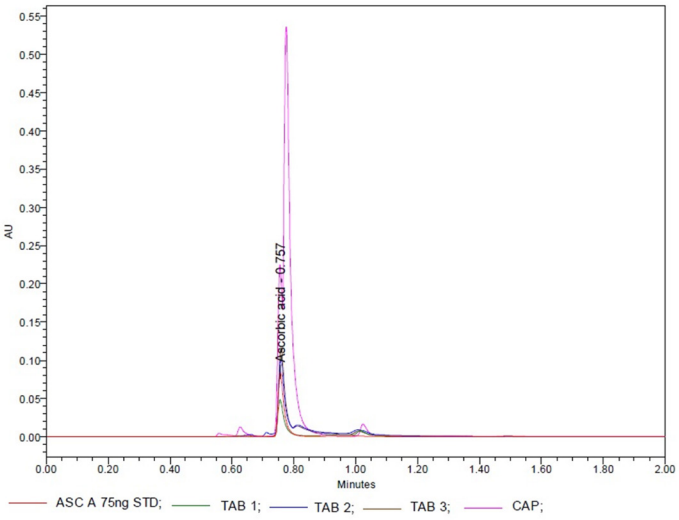

Figure 2: Overlay UPLC chromatogram of standard ASAC, Tablet-1, Tablet-2, Tablet-3, and Capsule-1.

Table 1: ASAC content in marketed formulation labeled claim.

		UPLC Method		UV Method	
Dosage forms	**Labeled claim. mg/Tab**	**Observed content**	**% W/W**	**Observed content**	**% W/W**
Tab	500	512.20±1.374	102.617±0.613	492.35±0.719	98.416±0.142
Tab 2	1000	1076.80±1.741	107.768±0.781	1079.38±0.580	107.898±0.191
Tab 3	1000	1046.05±5.636	100.679±0.498	1026.43±0.813	102.532±0.400
Cap 1	150	152.191±2.656	101.246±0.693	149.098±0.239	99.32±0.229

Table 2: Comparison with Reported Analytical Methods.

Sl. No.	Techniques	Column	Run time	Linearity	Rt	LOD LOQ	% Recovery	% RSD Intraday/ Interday	References
1	HPLC	C18	8 Min	1.0-12 µg/mL	3.5 min	LOD: 0.05 µg/mL LOQ: 0.17 µg/mL	95.4-101.5%	0.38%/1.22%	53
2	RP-HPLC	C18	40 Min	101-304 µg/mL	2.4	NR	98-102%	0.19%/0.75%	54
3	HPLC-ED	C18	8 min	12.5-1000 µM	5.4 min	--	98-104%	NR	41
4	HPLC	C18	18 Min	1-100 µg/mL	12.8 min	LOD: 0.1 µg/mL LOQ: 0.3 µg/mL	99%	4.6%/---	55
	HPLC	C18	14 Min	10-100 µg/mL	8.2 min	LOD: 0.03 µg/mL LOQ: 0.1 µg/mL	99%	1.51%/1.55%	56
6	HPLC	C18	--	0.25-1.5 mg/mL	3.5 min	LOD: 1.95 µg/mL LOQ: 6.5 µg/mL	99-102%	NR	57
7	HPLC	C18	10 min	0.1-2.5 mg/mL	0.99 min	LOD: 0.05 mg/mL LOQ: 0.1 mg/mL	95-100%	1.78%/11.5%	58
8	UPLC	C18	10 min	0.006-0.1 mg/ mL	1.67 min	NR	NR	NR	59
9	UPLC	HSS T3	3 MIN	0.5-25 µg/mL	1.87 min	NR	95.6%	1.75%/4.22%	60
10	HPLC	C18	15 Min	0-300 µg/mL	4.01min	LOD: 0.049 µg/mL LOQ: 0.149 µg/mL	98-100%	2.2%/2.4%	40
11	UPLC	C18	4 Min	0-50 µg/mL	1.56 Min	LOD: 0.024 µg/mL LOQ: 0.073 µg/mL	99-100%	1.7%/1.9%	40
12	HPLC	C18	5 Min	12.5-100 µg/mL	3.148 Min	LOD: 8.078 µg/mL LOQ: 34.90 µg/mL	105%	9.34%/---	(21)
13	UV Spectrophotometer	--	--	12.5-100 µg/mL		LOD: 1.35 µg/mL LOQ: 32.86 µg/mL	95%	NR	21
14	HPTLC	--	--	25-1200 ng/band range.	--	LOD: 8.630 ng/band LOQ: 25.89 ng/band	98.2-99.1%	0.97%/1.30%	61
15	HPTLC	--	--	200-800 ng/band	--	LOD: 8.22 ng/mL LOQ: 15.21 ng/mL	99.5-101.8%	0.68%/0.81%	62
16	Spectrophotometric method	--	--	40-320 µg/cm^3	--	--	NR	NR	63
17	HPLC	C18	13 Min	5-500 µg/mL	2.14 Min	LOD: 7.89 µg/mL LOQ: 23.89 µg/mL	98.3-103.7%	0.74%/1.38%	64
18	HPLC	C18	14 Min	2.5-80 µg/mL	2.497 Min	NR	98.7-101.6%	1.22%/1.33%	65
19	HPLC	C18	6.5 Min	0.1-100 µg/mL	3.4 Min	LOD: 0.02 µg/mL LOQ: 0.10 µg/mL	98.7-102.2%	1.92%/1.28%	66
20	Proposed UPLC METHOD	C8	2 min	25-125 ng/mL	0.75 min	LOD: 8 ng/mL LOQ:24.52 ng/mL	97.5-104%	0.27%/0.10%	CI*

*CI=Current investigation.

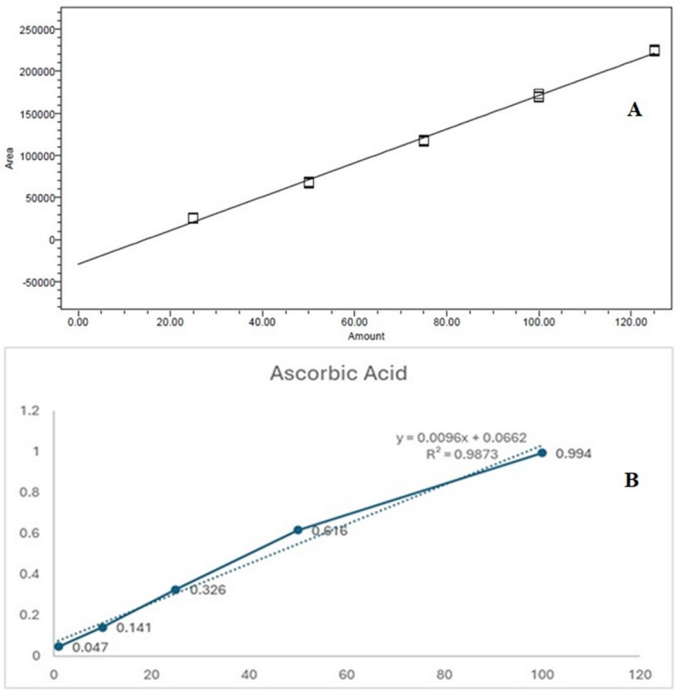

Figure 3: Calibration curves of ASAC (A) UPLC and (B) UV.

showed a strong correlation between ASAC concentration and UPLC and UV response.

For the proposed and developed method RT, peak area, USP tailing, and K Prime results yielded were 0.757±0.001, 27181.830±1375.087, 1.787±0.018, and 6.569±0.008, respectively. These obtained results determined that the developed UPLC method was suitable for assessing the ASAC content in the pharmaceutical formulations containing ASAC. The acquired findings of RT, peak area, USP tailing, and K Prime were within the acceptable range.[36] Hence, the findings for assessing ASAC were reliable.

The precision assessment data for the developed UPLC method were calculated as percentage of Relative Standard Deviation (%RSD) and values are presented. The %RSD of ASAC for the proposed UPLC method was 0.196, 0.788, and 0.273 at low, medium, and high concentrations, respectively, for precision within the day. For precision in between the day, the % RSDs of ASCA for the UPLC method at low, medium, and high concentration, respectively, were estimated to be 1.173, 0.196, and 0.110. The obtained % RSD of ASAC were within the acceptable range.[48] These results indicate that the developed UPLC method is precise for assessing ASAC in Pharmaceutical formulations.

The % recovery of ASAC for the developed UPLC method was determined to be 102.66%, 97.49%, and 105.27%, respectively at low, medium, and high concentration levels. The obtained % recoveries of ASAC were within the acceptable range.[48] These % recovery findings showcase the accuracy of the proposed UPLC method for assessing the ASAC in pharmaceutical formulations.

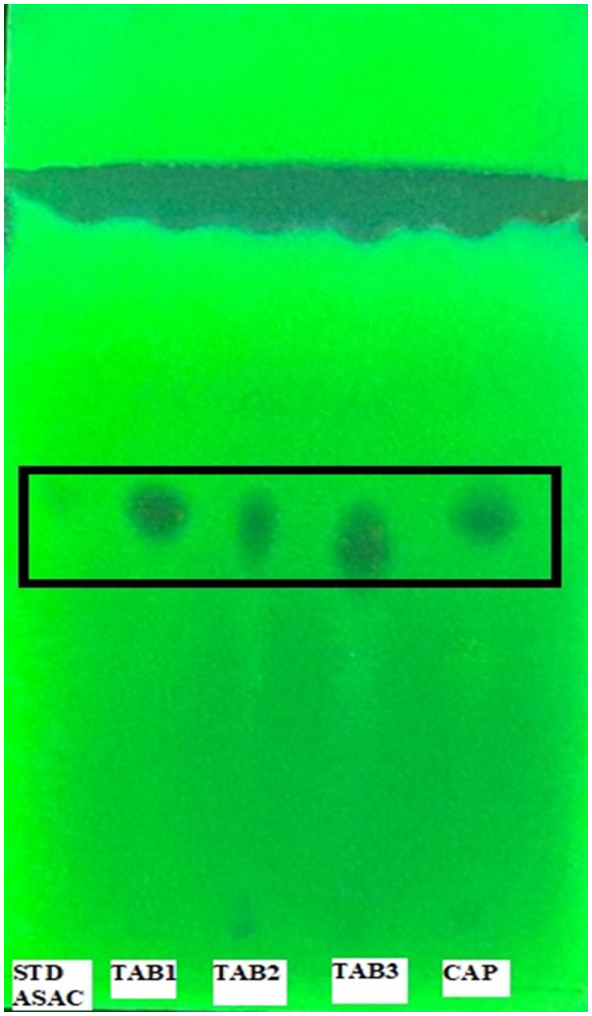

Figure 4: Developed TLC plate for the analysis of ASAC using solvent system Acetone-toluene-formic acid (6: 3: 1, v/v/v).

The robustness of the method was evaluated by making few changes in flow rate and mobile phase solvent. The %RSD for the robustness assessment was assessed to be 0.42-2.33% and 0.10-0.218% for mean peak area and retention time for flow rate of the developed method. For mobile phase composition, the %RSD for the mean peak area and retention time was 0.13-0.60% and 0.11-0.21%, respectively. The robustness of the developed UPLC method for assessing ASAC in pharmaceutical formulations was demonstrated by little fluctuations in the %RSD of retention time of ASAC.

The LOD and LOQ results of the method were calculated. For evaluating ASCA, LOD and LOQ were estimated to be 8.09±0.081, 24.52±0.277 ng/mL, and 1±0.016, 17.63±0.14 µg/mL for UPLC and UV methods, respectively. LOD and LOQ values confirmed the methods' sensitivity for the assessment of ASAC in pharmaceutical formulations.

The method specificity was determined by comparing the PDA spectrum index plot of ASAC in pharmaceutical formulations: Tablet-1, Tablet-2, Tablet-3 and Capsule-1 with those of standard ASAC. Figure 1 shows the PDA spectra of ASAC and ASAC in

pharmaceutical formulations. For the proposed UPLC method ASAC in standard and ASAC in Tablet-1, Tablet-2, Tablet-3 and Capsule-1 showed maximum response at 244.3 nm. The UPLC methods specificity was demonstrated by the matching PDA spectra, Rt values, and wavelength of ASAC in standard ASCA in Tablet-1, Tablet-2, Tablet-3 and Capsule-1.

Assessment of ASCA in marketed formulations

The developed method was used for the assessment of ASAC content in Tablet-1, Tablet-2, Tablet-3 and Capsule-1. For the developed UPLC method peaks were compared at Rt = 0.757±0.001 for the UPLC chromatogram of ASAC from the Tablet-1, Tablet-2, Tablet-3 and Capsule-1 with that of standard ASAC. The chromatographic parameters of ASAC in Tablet-1, Tablet-2, Tablet-3 and Capsule-1 are displayed in Figure 2, ASCA peaks were identical is standard and formulations. The detection of additional peaks in Tablet-1, Tablet-2, Tablet-3 and Capsule-1 showed its effectiveness in the assessment of ASAC in the presence of impurities/excipients/other phytoconstituents.

The calibration curve of ASAC was utilized to calculate the amount of ASAC in different formulations, and the results are showcased in Table 1. The quantity detected in Tablet-1, Tablet-2, Tablet-3 and Capsule-1 was assessed to be 512.20±1.374 mg/Tab, 1076.80±1.741 mg/Tab, 1046.05±5.636 mg/Tab and 1046.05±5.636 mg/Cap, respectively by developed UPLC method, respectively. However, the amount of ASAC in Tablet-1, Tablet-2, Tablet-3 and Capsule-1 was assessed to be 492.35±0.719 mg/Tab, 1079.38±0.580 mg/Tab, 1026.43±0.813 mg/Tab and 149.098±0.239 mg/Cap, respectively by developed UV method, respectively. The amount of ASAC was computed as higher in Tablet-1, Tablet-3, and Capsule-1 by the developed UPLC method compared to the UV method. Whereas the amount of ASAC was computed as higher in Tablet-2 by the UV method compared to the UPLC method. In summary, our reports disclosed that the proposed and developed UPLC method may be employed to evaluate ASAC in different foods and formulations with ASAC as one of the components.

TLC identification

Well-resolved ascorbic acid spotted after TLC plate development spotted at Retardation factor (R_f) 0.52 at 254 nm (Figure 4) in different pharmaceutical formulations.

DISCUSSION

The proposed UPLC technique for the detection and quantification of ASAC was evaluated against the published analytical methods. Table 2 displays the findings of the comparison. Parameters for validation of the method LOD, LOQ, and Linearity apart from other parameters like runtime, retention time, mobile phase, and detection wavelength were compared with the other methods published.

The proposed UPLC and UV method was found to be linear in the range of 25-125 ng/mL and 1-100 µg/mL, respectively. The amount of ASAC was computed as higher in Tablet-1, Tablet-3, and Capsule-1 by the developed UPLC method compared to the UV method. Whereas the amount of ASAC was computed as higher in Tablet-2 by the UV method compared to the UPLC method. The UPLC method completed the analysis of ASAC within 2 min whereas UV method needed much more time and solvent. These findings indicated that UPLC method is highly sensitive, fast, and less time and solvent consuming compared to the UV method. On the other hand, the proposed UV method was found to be superior compared to other reported UV methods.[21,63]

The linear behavior of the present UPLC method was found to be 25-125 ng/mL which was found to be superior among other analytical techniques reported. In addition to the current technique the other two techniques that reported a lower linearity range were the HPTLC method 25-1200 ng/band range[61] and 200-800 ng/band range.[62] Although they were found to be inferior to the current method but were comparable. When comparing the run time, the current UPLC method detected the analytes in 2 min which was found to be the shortest time among all the reported methods for the estimation of ASAC although there was one reported method with a runtime of 3 min,[60] that was the only one closer to the current method, therefore the current method is found to be timesaving. The currently developed method had a retention time of 0.75 min which was found to be the least time for the detection of the analytes within 2 min of analysis. The lowest reported in the literature was 0.99 min in a HPLC Method for Analysis of ASAC in locally packed Juices of Pakistan.[58] The current method reported a LOD of 8 ng/mL, and an LOQ of 24.52 ng/mL, which is found to be excellent among all the methods in literature. Another reported method was HPTLC for showing closer values was LOD 8.22 ng/mL LOQ 15.21 ng/mL in freeze-dry pomegranate juice and herbal formulation.[62] The linearity range of the other reported methods including: HPLC, HPTLC and UPLC were found to be inferior to the current UPLC method and similar and superior results to the current UV method.[64-66] However, the precision of the current UPLC method were found to be much superior whereas accuracy were inferior from few reported methods. Overall, the current UPLC method was superior to reported HPLC, HPTLC, UPLC and UV methods for the estimation of ASAC. All the validation parameters precision, specificity, accuracy, robustness, LOD, and LOQ were within the ICH guidelines limits, in summary, the present UPLC method to estimate ASAC was found to be leading among all published methods of ASAC analysis.

CONCLUSION

The current method aims to come up with a simple, sensitive, and cost-effective UPLC technique for the estimation of ASAC in different pharmaceutical preparations (chewable tablets, effervescent tablets, and capsules). The validation of this UPLC method was done following the ICH guidelines for linearity, precision, LOQ, accuracy, sensitivity, and robustness. The method showcased linearity, high sensitivity, simplicity, robustness, and cost-effectiveness based on their low retention time, flow rate, and low injection volume. The present UPLC method demonstrated superiority compared to other reported analytical methods of ASAC analysis making it an attractive alternative for the estimation of ASAC in different pharmaceutical formulations. Furthermore, linear regression analysis of the UV-visible spectrophotometric method was done, and it was found to be linear and sensitive based on their regression coefficient LOD, and LOQ values. The qualitative identification of the ASAC was done by TLC.

ACKNOWLEDGEMENT

The authors are thankful to Mohammed Al-Mana College for Medical Sciences for providing instrumentation facility to conduct this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ASAC: Ascorbic Acid; **UPLC:** Ultra-Performance Liquid Chromatography; **UV:** UV Spectrophotometry (or UV method); **HPLC:** High-Performance Liquid Chromatography; **TLC:** Thin-Layer Chromatography; **PDA:** Photo Diode Array detector; **ICH:** International Conference on Harmonization; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RT/Rt:** Retention time; **R2:** Correlation coefficient; **%RSD:** percentage of Relative Standard Deviation; **K Prime:** Capacity factor (or retention factor); **FA:** Formic Acid; **HPTLC:** High-Performance Thin-Layer Chromatography; **RP-HPLC:** Reversed-Phase High-Performance Liquid Chromatography; **BP:** British Pharmacopoeia; **USP:** United States Pharmacopoeia; **Ph. Eur.:** European Pharmacopoeia; **v/v/v:** volume/volume/volume; **mg/Tab:** milligrams/Tablet; **mg/Cap:** milligrams/Capsule; **CI:** Current investigation.

REFERENCES

1. Jassim SA, Mahmood JM, Imran NA, Ali AS. Optimum conditions for ascorbic acid determination in three Iraqi citrus using HPLC technique. Baghdad Sci J. 2014;11(3):1233-42. doi: 10.21123/bsj.2014.11.3.1233-1242.
2. Aruoah MK, Al-Jowari SA. The effects of zinc and vitamin C supplementation on the glycemic profile in type 2 diabetic patients. Iraqi J Sci. 2022: 70-6. doi: 10.24996/ijs.2022.63.1.8.
3. nih. gov/factsheets/VitaminC-HealthProfessional. Vitamin D. J Diet Suppl. od. 2017. Fact sheet for health professionals.
4. Murad S, Grove D, Lindberg KA, Reynolds G, Sivarajah A, Pinnell SR. Regulation of collagen synthesis by ascorbic acid. Proc Natl Acad Sci U S A. 1981;78(5):2879-82. doi: 10.1073/pnas.78.5.2879, PMID 6265920.
5. Shao LiHua SL, Li JingTao LJ, Guo Shuai GS, Wang Shu E. WS, Guo DongMei GD. Determination of reduced and total vitamin C in beverage by high performance liquid chromatography.
6. Eoy M, Drug GK. Bethesda: American Society of Health System Pharmacists, Incorporated; 2000. p. 3328-30.
7. Pugliese PT, Lampley CB. Biochemical assessment of the anti-aging effects of cosmetic products. J Appl Cosmetol. 1985;3:129-38.
8. Carr AC, Maggini S. Vitamin C and immune function. Nutrients. 2017;9(11):1211. doi: 10.3390/nu9111211, PMID 29099763.
9. Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease. Nutr Clin Care. 2002;5(2):66-74. doi: 10.1046/j.1523-5408.2002.00005.x, PMID 12134712.
10. Chang SK, Ismail A, Daud ZA. Ascorbic acid: properties, uses and determination./Sui. In: Encyclopedia of Food and Health. Kiat Chang and Ismail, Amin and Mat Daud, Zulfitri Azuan. Ascorbic acid: properties, determination and uses. London, United Kingdom: Academic Press. ISBN 0123849470; 2016. p. 275-84/9780123849472; EISBN: 9780123849533.
11. Combs Jr GF, McClung JP. The vitamins: fundamental aspects in nutrition and health. Academic press; 2016.
12. Desai AP, Desai S. UV spectroscopic method for determination of vitamin C (ascorbic acid) content in different fruits in south Gujarat Region. Int J Environ Sci Nat Resour. 2019;21(2):41-4.
13. British Pharmacopoeia. Commission. British pharmacopoeia, Her Majesty Stationary Office: London. UK, 2018;1:203-4.
14. USP 42. In: United States pharmacopoeia; The United States pharmacopeial convention: Rockville, MD, USA; 2018. p. 377-8.
15. European Pharmacopoeia Commission; European Directorate for the Quality of Medicines and Healthcare. European pharmacopoeia. Council of Europe; 2010.
16. Joint Formulary Committee, editor. British National Formulary. Pharmaceutical Press; 2012.
17. Baker EM, Hodges RE, Hood J, Sauberlich HE, March SC. Metabolism of ascorbic-1-14C acid in experimental human scurvy. Nutr (Burbank Los Angeles Cty Calif). 1993. 1969;9(1):75-84.
18. Vitamin C. Requirement of human adults experimental study of vitamin-C deprivation in man: a preliminary report by the vitamin C subcommittee of the accessory food factors committee, Medical Research Council. Lancet. 1948;1(6510):853-8. PMID 18866290.
19. Arya SP, Mahajan M, Jain P. Photometric methods for the determination of vitamin C. Anal Sci. 1998;14(5):889-95. doi: 10.2116/analsci.14.889.
20. Lyne G, Scurvy MJ (Vitamin C Deficiency); 2024. Available from: https://emedicine.medscape.com/article/125350-clinical?form=fpfUpdated.
21. Bonde S, Bhadane R, Walke S, Gupta N. Determination of vitamin-C from formulated drug by HPLC and UC/VIS-spectrophotometry techniques. Int J Pharm Bio Med Sci. 2015;4(5):7-15.
22. Albuquerque B, Lidon FC, Leitão AE. Ascorbic acid quantification in melon samples-The importance of the extraction medium for HPLC analysis. Gen Appl Plant Physiol. 2005;31(3-4):275-51.
23. Mazurek A, Pankiewicz U. Changes of dehydroascorbic acid content in relation to total content of vitamin C in selected fruits and vegetables. Acta Sci Pol Hortorum Cultus. 2012;11(6).
24. Mitic SS, Kostic DA, Naskovic- okic DC, Mitic MN. Rapid and reliable HPLC method for the determination of vitamin C in pharmaceutical samples. Trop J Pharm Res. 2011;10(1). doi: 10.4314/tjpr.v10i1.66549.
25. Api AM, Belsito D, Biserta S, Botelho D, Bruze M, Burton GA et al. RIFM fragrance ingredient safety assessment, 2,4-dimethyl-4-phenyltetrahydrofuran, CAS registry number 82461-14-1. Food Chem Toxicol. 2020; 144 Suppl 1:111634. doi: 10.1016/j.fct.2020.111634, PMID 32781225.
26. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: a review. Arab J Chem. 2017;10:S1409-21. doi: 10.1016/j.arabjc.2013.04.016.
27. Khorshed AA, Khairy M, Banks CE. Electrochemical determination of antihypertensive drugs by employing costless and portable unmodified screen-printed electrodes. Talanta. 2019;198:447-56. doi: 10.1016/j.talanta.2019.01.117, PMID 30876585.
28. Duan C, Wu Y, Yang J, Chen S, Pu Y, Deng H. Simultaneous determination of cortisol, cortisone, and multiple illicit drugs in hair among female drug addicts with LC-MS/MS. Molecules. 2021;26(2):516. doi: 10.3390/molecules26020516, PMID 33478156.
29. Terzopoulou Z, Papageorgiou M, Kyzas GZ, Bikiaris DN, Lambropoulou DA. Preparation of molecularly imprinted solid-phase microextraction fiber for the selective removal and extraction of the antiviral drug abacavir in environmental and biological matrices. Anal Chim Acta. 2016;913:63-75. doi: 10.1016/j.aca.2016.01.059, PMID 26944990.
30. Arya SP, Mahajan M, Jain P. Non-spectrophotometric methods for the determination of vitamin C. Anal Chim Acta. 2000;417(1):1-14. doi: 10.1016/S0003-2670(00)00909-0.
31. Moser U, Bendich A. Vitamin C. In: Machlin LJ, editor. Handbook of vitamins. New York: Marcel Dekker; 1991. p. 195-232.
32. Jaffe GM. Handbook of vitamins. New York: Marcel; 1984.

33. Ball GF. Water-soluble vitamin assays in human nutrition. Springer; 2012.
34. Thirumagal S, Rajagopalan P. Enzymatic and chromatographic methods for quantification of ascorbic acid in food products and plant extracts. Food Chem. 2020;314:126161. doi: 10.1016/j.foodchem.2019.126161.
35. Liu Q, Zhang H. Recent developments in chromatographic methods for the determination of ascorbic acid in biological and pharmaceutical samples. TrAC Trends Anal Chem. 2019;118:75-85. doi: 10.1016/j.trac.2019.05.001.
36. Sofia P, Tania-Ştefania C, Miruna GC, Antonia-Claudia V, Luminita P, Ariana V. Vitamin C determination in foods. Titrimetric methods-A review. J Agroaliment Processes Technol. 2024;30(4):471-6.
37. Braulio GR, Stéphanie R, Francis F. Spectrophotometric method for fast quantification of ascorbic acid and dehydroascorbic acid in simple matrix for kinetics measurements. Food Chem. 2016;211:583-9. doi: 10.1016/j.foodchem.2016.05.107, PMID 27283671. ff10.1016/j.foodchem.2016.05.107ff. ffhal-01533897.
38. Wu F, Xu F, Liu W, Chen S, Luo H, Cheng N et al. A high-performance liquid chromatography with electrochemical detection method developed for the sensitive determination of ascorbic acid: validation, application, and comparison with titration, spectrophotometric, and high-performance liquid chromatography with diode-array detection methods [application]. Foods. 2023;12(16):3100. doi: 10.3390/foods12163100, PMID 37628099.
39. Shekhovtsova TN, Muginova SV, Luchinina JA, Galimova AZ. Enzymatic methods in food analysis: determination of ascorbic acid. Anal Chim Acta. 2006; 573-574:125-32. doi: 10.1016/j.aca.2006.05.015, PMID 17723515.
40. Klimczak I, Gliszczyńska-Świgło A. Comparison of UPLC and HPLC methods for determination of vitamin C. Food Chem. 2015 May 15;175: 100-5. doi: 10.1016/j.foodchem.2014.11.104, PMID 25577057.
41. Gazdik Z, Zitka O, Petrlova J, Adam V, Zehnalek J, Horna A, *et al.* Determination of vitamin C (ascorbic acid) using high performance liquid chromatography coupled with electrochemical detection. Sensors (Basel). 2008;8(11):7097-112. doi: 10.3390/s8117097, PMID 27873917.
42. Li Z, Zhao L. Chromatographic techniques in the analysis of ascorbic acid in commercial and pharmaceutical formulations. TrAC Trends Anal Chem. 2019;116:57-66. doi: 10.1016/j.trac.2019.04.001.
43. Guntarti A, Hutami EN. Validation and vitamin C testing in crystal guava (*Psidium guajava* L.) with variations of origin with the HPLC method (high performance liquid chromatography). Int J Chem. 2019;11(1):52. doi: 10.5539/ijc.v11n1p52.
44. Desai AP, Desai S. UV spectroscopic method for determination of vitamin C (ascorbic acid) content in different fruits in south Gujarat Region. Int J Environ Sci Nat Resour. 2019;21(2):41-4.
45. Pyka-Pająk A, Dołowy M, Parys W, Bober K, Janikowska G. A simple and cost-effective TLC-densitometric method for the quantitative determination of acetylsalicylic acid and ascorbic acid in combined effervescent tablets. Molecules. 2018;23(12):3115. doi: 10.3390/molecules23123115, PMID 30487409.
46. Ganea M, Ghitea TC, Moisa C, Bota S. Thin-layer chromatographic method for identifying vitamin C in fruits and drugs.
47. Ahmad W, Yusuf M, Ahmad A, Hassan YA, Amir M, Wahab S. Development and validation of ultra performance liquid chromatography (UPLC) method for the quantitative estimation of caffeine in non-alcoholic soft and energy drinks. J AOAC Int. 2022 Jul 1;105(4): 1146-52. doi: 10.1093/jaoacint/qsac016, PMID 35148411.
48. Guideline on validation of Analytical Procedures—text and Methodology International conference on Harmonization (ICH), Q2. Vol. R2. Geneva, Switzerland; 2023.
49. Ahmad W. Box-Behenken-Supported Development and Validation of UPLC Method for the Estimation of eugenol in Syzygium aromaticum, Cinnamomum tamala, and Myristica fragrance. Separations. 2023;10(4):248. doi: 10.3390/separations10040248.
50. Ahmad W, Ahmad A, Ghosn SA, Ali A, Ansari MN, Wahab S, *et al.* Simultaneous determination of theobromine (THEOB), theophylline (THEOP) and caffeine (CAF) in commercially available food products using UPLC technique. Acta Chromatographica. 2025;37(4):476-86. doi: 10.1556/1326.2024.01292.
51. Ahmad W, Hassan YA, Ahmad A, Suroor M, Sarafroz M, Alam P, *et al.* A simple stability-indicating UPLC method for the concurrent assessment of paracetamol and caffeine in pharmaceutical formulations. Separations. 2023;10(1):50. doi: 10.3390/separations10010050.
52. Ahmad W, Zaidi SM, Mujeeb M, Ansari SH, Ahmad S. HPLC and HPTLC methods by design for quantitative characterization and *in vitro* anti-oxidant activity of polyherbal formulation containing Rheum emodi. J Chromatogr Sci. 2014 Sep 1;52(8): 911-8. doi: 10.1093/chromsci/bmt123, PMID 23978770.
53. Maia AM, Baby AR, Yasaka WJ, Suenaga E, Kaneko TM, Velasco MV. Validation of HPLC stability-indicating method for vitamin C in semisolid pharmaceutical/cosmetic preparations with glutathione and sodium metabisulfite, as antioxidants. Talanta. 2007;71(2):639-43. doi: 10.1016/j.talanta.2006.05.006, PMID 19071353.
54. Sridevi S, Vijayakumar R, Nalini CN. Method development and validation for the simultaneous estimation of ascorbic acid, phenylephrine HCl, paracetamol and levocetirizine HCl using RP-HPLC. Res J Pharm Technol. 2020;13(4):1913-8. doi: 10.5958/0974-360X.2020.00344.3.
55. Fontannaz P, Kilinç T, Heudi O. HPLC-UV determination of total vitamin C in a wide range of fortified food products. Food Chem. 2006;94(4):626-31. doi: 10.1016/j.foodchem.2005.02.012.
56. Hu L, Li L, Luo Z, Yang J, Liu W. Determination of trace vitamin C by ion-pair HPLC with UV detection in calcium gluconate and vitamin C compound oral solution. J Chromatogr Sci. 2012;50(2):102-7. doi: 10.1093/chromsci/bmr035, PMID 22298758.
57. Mitic SS, Kostic DA, Naskovic- okic DC, Mitic MN. Rapid and reliable HPLC method for the determination of vitamin C in pharmaceutical samples. Trop J Pharm Res. 2011;10(1). doi: 10.4314/tjpr.v10i1.66549.
58. Shafqat Ullah SU, Arshad Hussain AH, Javid Ali JA, Khaliqurrehman K, Asad Ullah AU. A simple and rapid HPLC method for analysis of vitamin-C in local packed juices of Pakistan. Middle East J Sci Res. 2012;12(8):1085-91.
59. Cotruţ R, Bădulescu L. UPLC rapid quantification of ascorbic acid in several fruits and vegetables extracted using different solvents. Agric Agric Sci Procedia. 2016;10:160-6. doi: 10.1016/j.aaspro.2016.09.047.
60. Medina-Lozano I, Bertolín JR, Zufiaurre R, Díaz A. Improved UPLC-UV method for the quantification of vitamin C in lettuce varieties (*Lactuca sativa* L.) and crop wild relatives (*Lactuca* spp.). J Vis Exp. 2020;(160):e61440. doi: 10.3791/61440, PMID 32716366.
61. Foudah AI, Alam P, Shakeel F, Alam A, Salkini MA, Alshehri S et al. A simple, cost-effective, and green HPTLC method for the estimation of ascorbic acid in solvent and ultrasound-assisted extracts of *Phyllanthus emblica, Capsicum annuum,* and *Psidium guajava*. Agronomy. 2022;12(5):1016. doi: 10.3390/agronomy12051016.
62. Alam P, Kamal YT, Alqasoumi SI, Foudah AI, Alqarni MH, Yusufoglu HS. HPTLC method for simultaneous determination of ascorbic acid and gallic acid biomarker from freeze dry pomegranate juice and herbal formulation. Saudi Pharm J. 2019;27(7):975-80. doi: 10.1016/j.jsps.2019.07.006, PMID 31997904.
63. Jagasia PV, Divyadarshani B. Spectrophotometric determination of vitamin C in pharmaceutical preparations using ammonium metavanadate. Asian J Res Chem. 2017;10(3):341-4. doi: 10.5958/0974-4150.2017.00057.8.
64. AlKaraki RN, Tarawneh SK, Haddadin RR, Aqil R, Alkasasbeh RM. A new RP-HPLC method for the simultaneous detection and quantification of minocycline and ascorbic acid in pure forms and combined pharmaceutical dosage forms. Results Chem. 2024;10(10):101701. doi: 10.1016/j.rechem.2024.101701.
65. Galal SA, Elzanfaly ES, Hussien EM, Amer EA, Zaazaa HE. Development of chromatographic methods to determine multivitamins formulation depending on their solubility and polarity: comparative study using three greenness assessment tools. BMC Chem. 2024;18(1):20. doi: 10.1186/s13065-024-01118-1, PMID 38281043.
66. ÖZEN G, NEMUTLU E. Green HPLC method for simultaneous determination of N-acetylcysteine and L-ascorbic acid in co-formulated pharmaceutical products. J Res Pharm. 2024; 28(5)(28(5)):1777-90. doi: 10.29228/jrp.850.

Cite this article: Ahmad W, Banu N, Ahmad A, Hassan YA, Surour M, Aleid RR, *et al*. Development and Validation of UPLC-PDA and UV Methods for the Quantitative Estimation of Ascorbic Acid (ASAC) in Pharmaceutical Formulations. Indian J of Pharmaceutical Education and Research. 2026;60(2):852-60.