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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(3):363-374

Development and validation of RP-HPLC method for the estimation of ascorbic acid in health drinks

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Ascorbic acid in Health drinks. Isocratic elution at a flow rate of 0.9ml/min was employed on a symmetry C_{18} (250x4.6mm, 0.5µ in particle size) at ambient temperature. The mobile phase consisted of Water with acetic acid: methanol 95:5% (v/v). The UV detection wavelength was 245 nm and 20µl sample was injected. The retention time for Ascorbic acid was 4.61+_ 0.22 min. The percentage RSD for precision and accuracy of the method was found to be less than the method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Ascorbic acid in Health drinks.

Key Words: Ascorbic acid, RP-HPLC, UV detection, Health drinks, recovery, linearity.

INTRODUCTION

Ascorbic acid is a reductone sugar acid with antioxidant properties. Its appearance is white to light-yellow crystals or powder, and it is water-soluble. Ascorbic acid is one form (vitamer) of vitamin C, and was historically the first chemical compound to be synthesized, and identified, as vitamin C. The name is derived from a- (meaning "no") and scorbutus (scurvy), the disease caused by a deficiency of vitamin C. Haworth and Szent-Györgyi when its structure was finally proven by synthesis.[1] Ascorbic acid is an enzyme cofactor in tyrosine oxidation.[2] It creates volatile compounds when mixed with glucose and amino acids.[3]

Ascorbate usually acts as an antioxidant by being available for energetically favourable oxidation. Ascorbic acid is easily oxidized and so is used as a reductant in photographic developer solutions (among others) and as a preservative. Ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives[4]. These compounds are water-soluble and thus cannot protect fats from oxidation[5].

Ascorbic acid Molecular formula- $C_6H_8O_6$ (fig.1) Molecular weight -176.12 g/mol.
IUPAC Name: (5*R*)-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one.

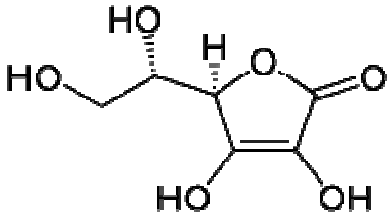


Fig.1 Chemical Structure of Ascorbic acid

EXPERIMENTAL SECTION

Chemicals and reagents

HPLC grade water with acetic acid, methanol and metaphosphoric acid was purchased from Merck Specalities Pvt. Ltd.

Instrumentation and analytical conditions

The analysis of drug was carried out on a Shimadzu HPLC system equipped with a reverse phase C_{18} column (250x4.6mm, 0.5µ in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on Shimadzu Chromatographic Software version 1.06. Isocratic elution with acetic acid: methanol 95:5% (v/v) was used at a flow rate of 0.9ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

ANALYSIS OF THE METHOD

Preparation of standard stock solution

10 mg of ascorbic acid was accurately weighed into a 100 ml standard flask and made up to the required volume with 0.56% $^{w}/_{v}$ of metaphosphoric acid solution to get a concentration of 100 µg/ml. From this solution 0.1 ml is taken and diluted to 10 ml for getting a concentration of 10 µg/ml. From that a concentration ranging 0.1-0.5 µg/ml was prepared.

Recording of a standard chromatogram

In the Shimadzu HPLC system, the chromatographic conditions were maintained. After a steady base line was obtained, 20 µl of the prepared working standard solution was injected and the chromatograms were recorded.(**Fig.2**)

Preparation of working standard solution

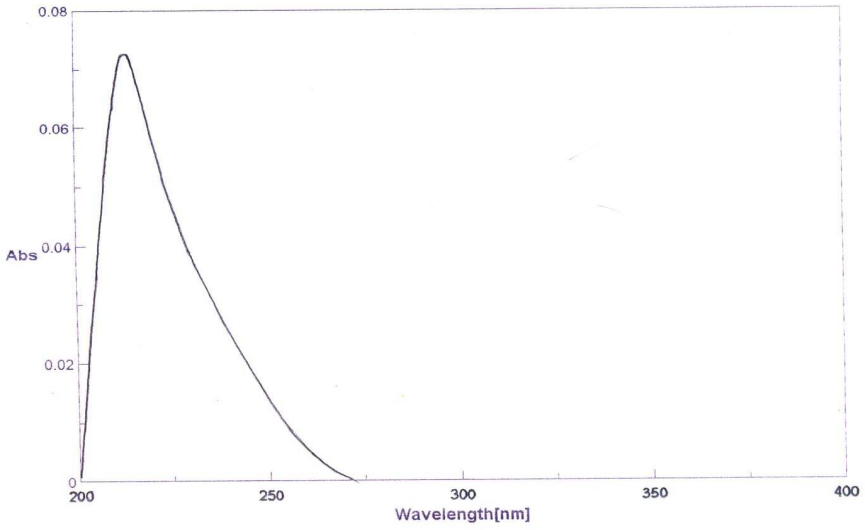
Serial concentration of 0.1-0.5 µg/ml was prepared from the stock concentration of 10 µg/ml,

from this 0.1, 0.2, 0.3, 0.4 and 0.5 was pipetted out into 10 ml standard flasks, and made up to the required volume with 0.56% w/v met phosphoric acid solution.

Extraction of ascorbic acid from marketed health drink

To extract ascorbic acid from fruit juices 0.56% w/v met phosphoric acid solution was added and centrifuged for 60 seconds at 2000 rpm and the filtrate was filtered and taken for the further studies.

FIG.2 Chromatogram of standard ascorbic acid



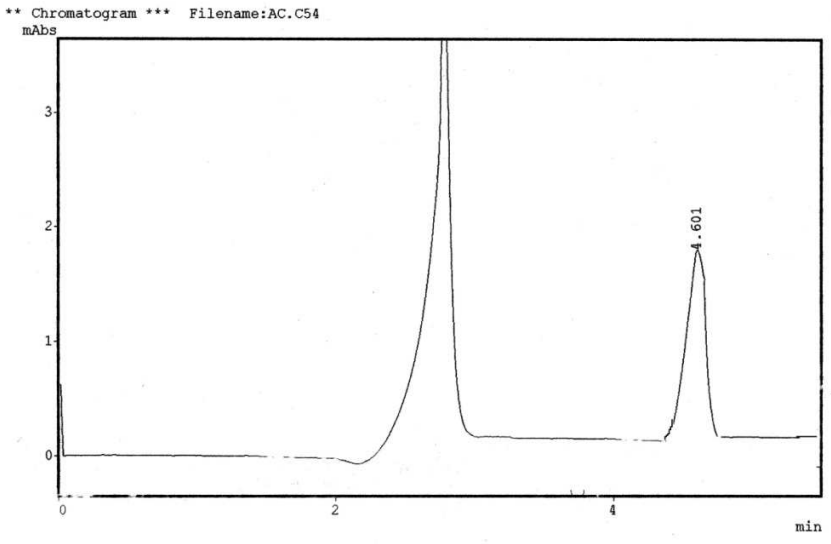


FIG.3. Chromatogram of 0.1 µg/ml standard ascorbic acid

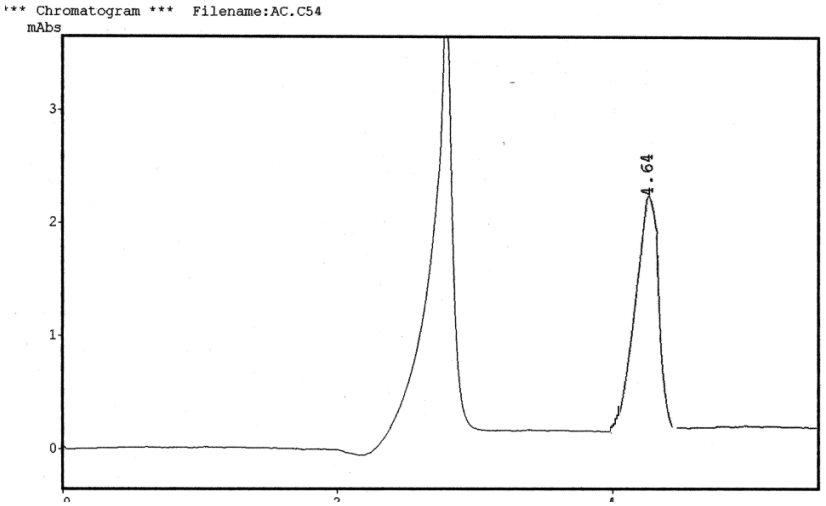


Fig4. Chromatogram of 0.2 µg/ml standard ascorbic acid

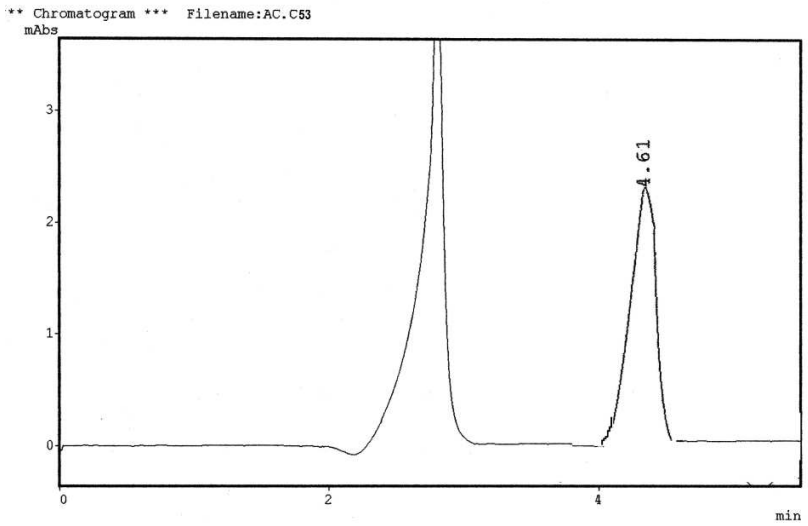


Fig.5. Chromatogram of 0.3 µg/ml standard ascorbic acid

Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines [6] . The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability.

Linearity and range

Ascorbic acid was found to be linear in the range of 0.1-0.6 mcg/ml. Calibration graph was plotted using peak area of standard peak areas Vs concentration of standard solutions.(**Fig.3-8**)

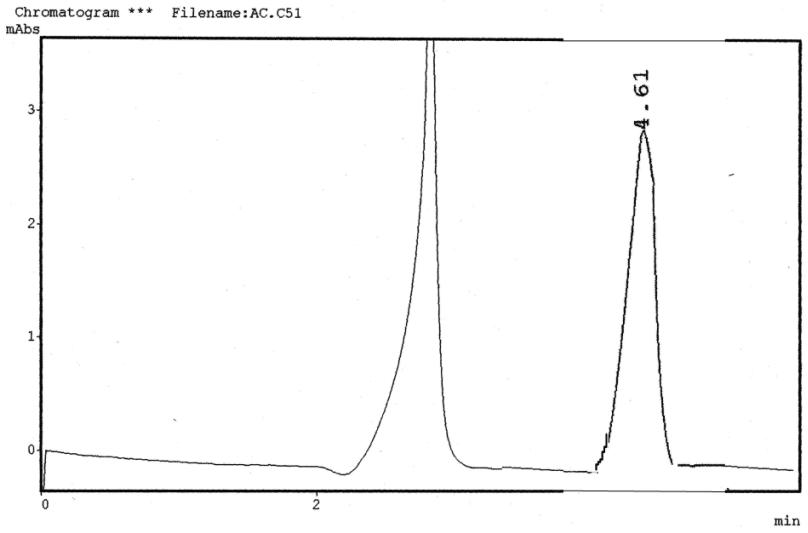


Fig.6. Chromatogram of 0.4 µg/ml standard ascorbic acid

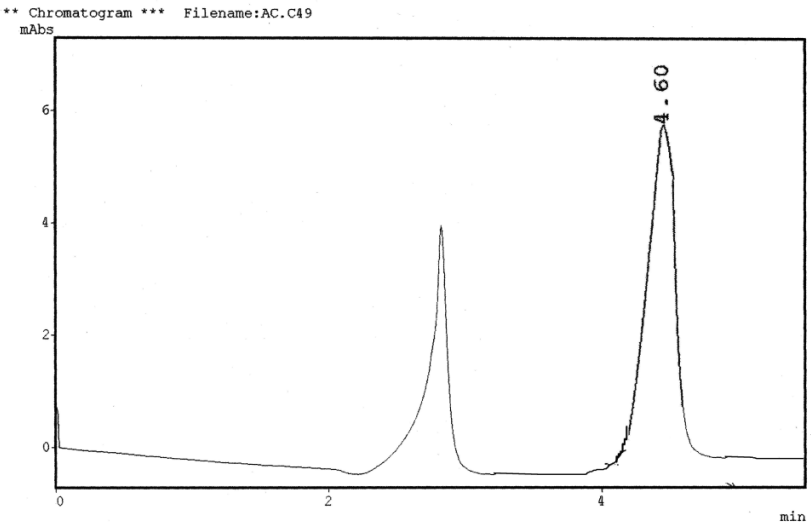


Fig 7. Chromatogram of 0.5 µg/ml standard ascorbic acid

Precision

a) Intra day precision
b) Inter day precision
c) Repeatability of injection

Intra day precision

This was done by carrying out the analysis of the standard ascorbic acid for three different concentrations on the same day. Each concentration was injected in triplicates and the peak areas were noted. The % RSD values were calculated.

Inter day precision
It was found out by carrying out the analysis for three days with three different concentrations of the linearity range. The % RSD values were calculated.

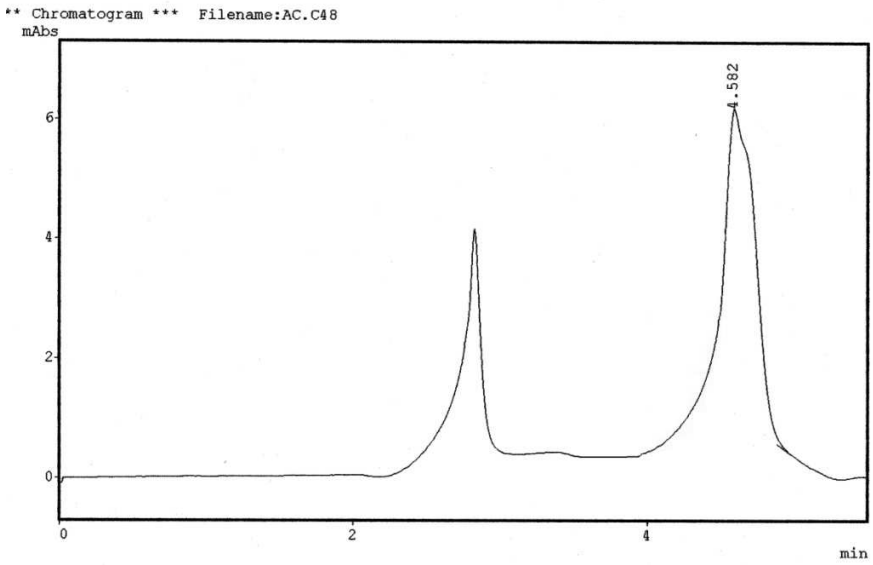


Fig 8. Chromatogram of 0.6 µg/ml standard ascorbic acid

Repeatability of Injection
A standard solution of ascorbic acid was injected 6 times and its % RSD values were calculated.

Accuracy
To study the reliability, suitability and accuracy of the method, recovery studies were carried out. To the formulation equivalent to 10 mg of ascorbic acid at the levels of 50% and 100% were added. Pure ascorbic acid was extracted with 0.56% v/v of phosphoric acid and made upto mark with same. The concentration of drugs present in resulting solution was determined using assay method, percentage recovery and percentage RSD were calculated.

Robustness
In order to demonstrate the robustness of the method, the following optimized conditions were slightly varied.

± 2% in ratios of methanol in mobile phase

The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters and hence the developed method is said to be robust.

Specificity
Conditions of HPLC method like percentage of organic solvent in mobile phase, and flow rate were changed. Although these changes were made, no additional peaks were found but there were some slight changes in retention times and peak shapes.

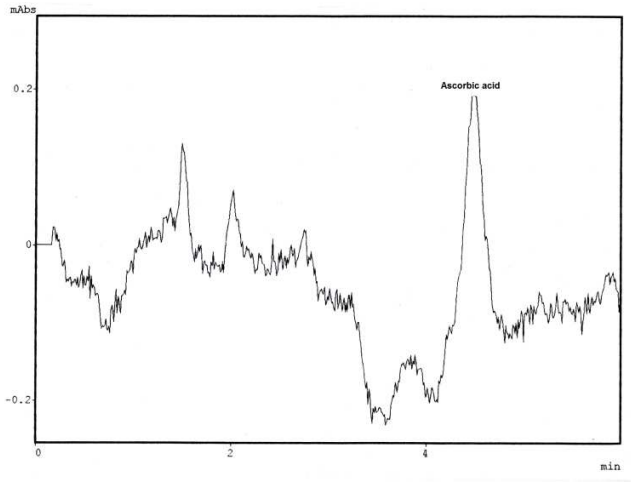


Fig 9.LOQ of Ascorbic acid

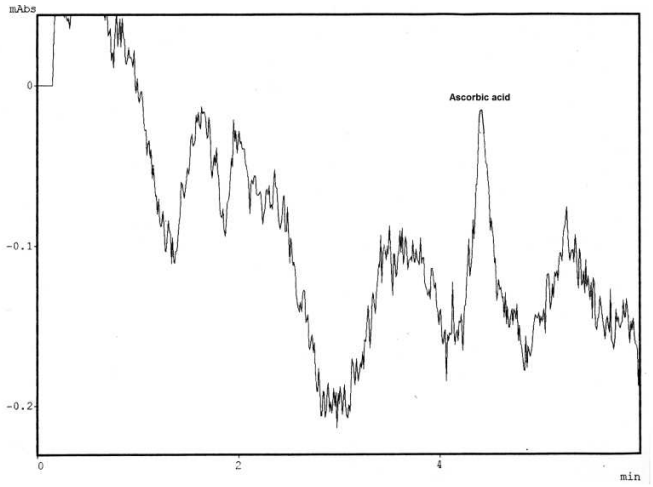


Fig 10.LOD of Ascorbic acid

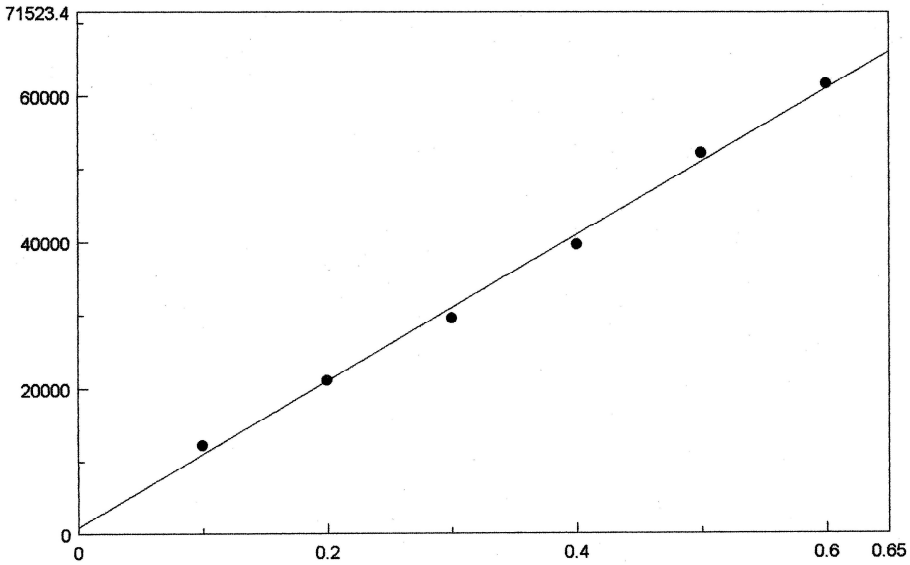


Fig 11.Calibration graph of standard Ascorbic acid

Stability

Sample solutions were subjected to stability studies under refrigerated and room conditions. Stabilities were studied by looking for any change in retention time, resolution and peak shape.

System suitability studies

System suitability parameters like number of theoretical plates (N), peak asymmetry factor (A_s), capacity factor (K^1), selectivity factor (α), resolution (R_s) were studied.

LOD and LOQ

The LOD and LOQ of ascorbic acid was found to be 0.01 and 0.1 mcg/ml respectively (Fig.9-10).

Linearity

The slope, intercept and correlation coefficient values were found to be 100166.8543, 993.2669 and 0.9979 respectively.(Fig.11)

TABLE 1: INTRADAY PRECISION

Day	Concentration (µg/ml)	Peak Area	% RSD*
1	0.1	12223	1.34
		12160	
		11916	
	0.2	21056	0.75
		20866	
		20746	
	0.3	29545	0.62
		29348	
		29179	

** Mean RSD of three observations.*

TABLE 2: INTERDAY PRECISION

Concentration (µg/ml)	Day	Peak area		% R.S.D*
0.2	1	21056	20932	1.25
	2	21365	21218	
	3	20885	20614	
0.3	1	29545	29483	1.14
	2	29865	29615	
	3	30412	29781	
0.4	1	39663	39584	0.78
	2	40236	39961	
	3	40256	39616	

**Mean RSD at six observations*

TABLE 3: REPEATABILITY OF INJECTION

Concentration (µg/ml)	Peak area	% RSD
0.5	52183	0.77
	51976	
	51865	
	51538	
	51318	
	51164	

TABLE 4: ACCURACY

Drug	% Recovery		% RSD*	
	50% level	100% level	50% level	100% level
Ascorbic acid	98.64	97.52	0.1528	0.3741

RESULTS AND DISCUSSION

STABILITY

The solution stored under room temperature was stable up to 24 hrs and under refrigeration up to 48hrs.

TABLE 5: SYSTEM SUITABILITY PARAMETERS

Drug	R_s	N	A_s	K^1	α
Ascorbic acid	8.95	2622	1.06	1.27	2.08

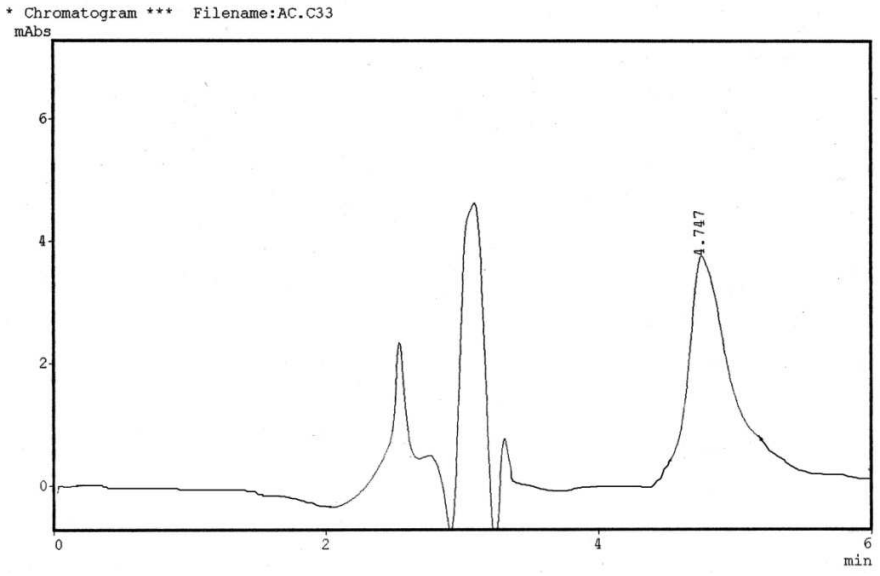


Fig 12. Chromatogram of ascorbic acid present in health drink
(*Within first week from the date of manufacturing****)***

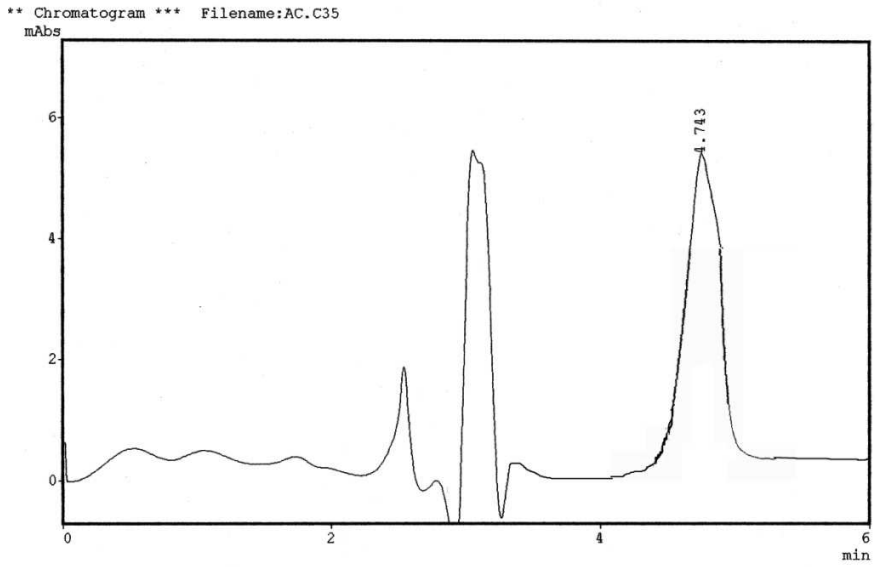


Fig 13. Chromatogram of ascorbic acid present in health drink
(At the end of 1st month)

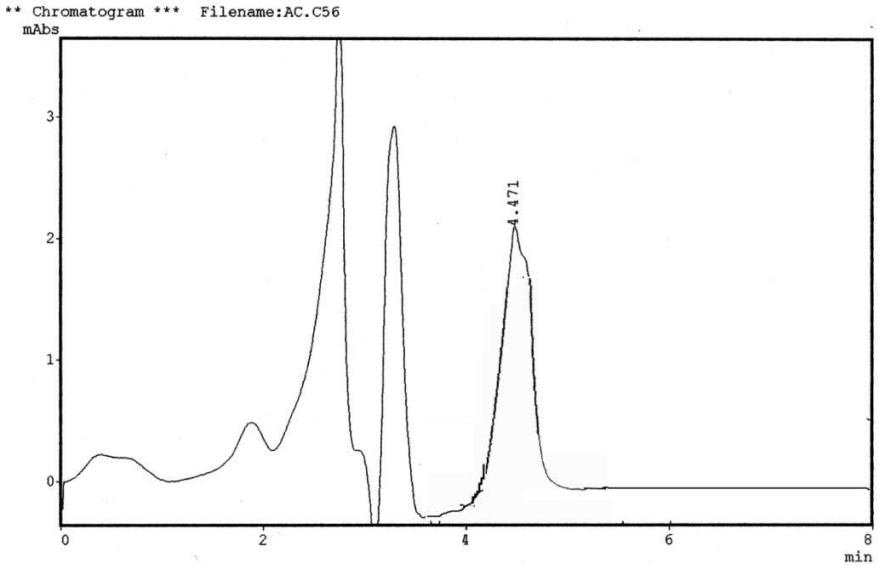


Fig 14. Chromatogram of ascorbic acid present in health drink
(At the end of 2nd month)

Estimation of ascorbic acid content in marketed health drinks at various time intervals

Preparation of sample

Marketed health drinks were obtained from the local markets of Coimbatore. Extraction of ascorbic acid content from marketed health drink, were done by the above mentioned procedure. The supernatant was taken and were injected for further studies. Chromatograms for ascorbic acid content in marketed health drink at various months were depicted in **figures 12-16**.

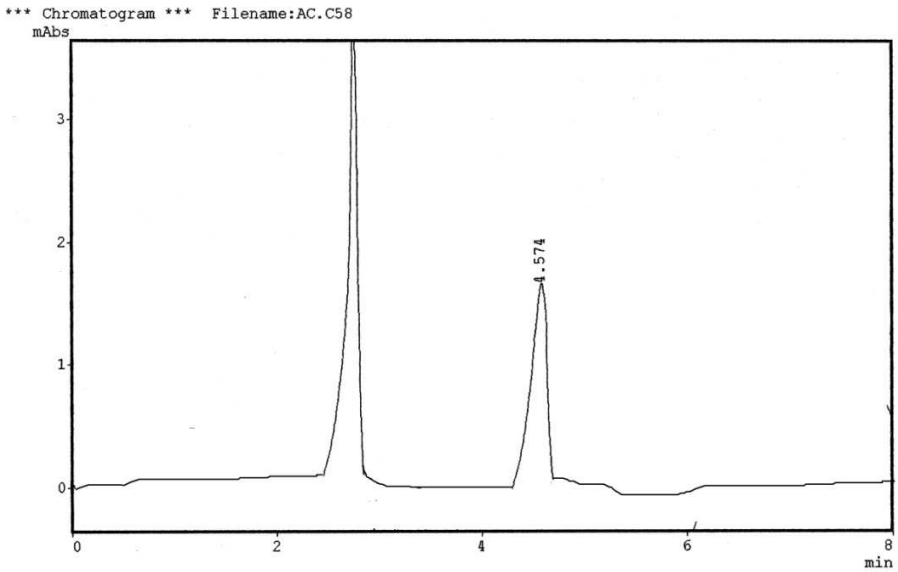


Fig 15. Chromatogram of ascorbic acid present in health drink
(At the end of 3rd month)

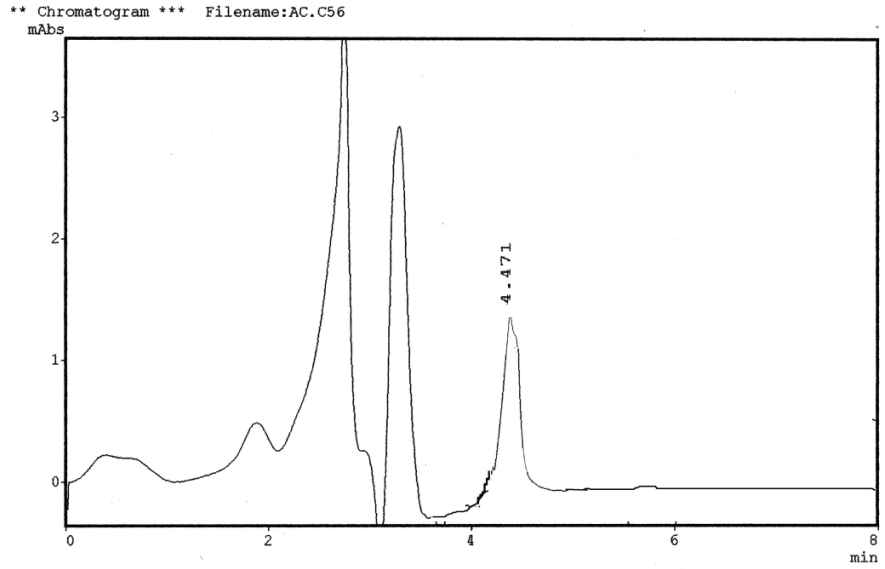


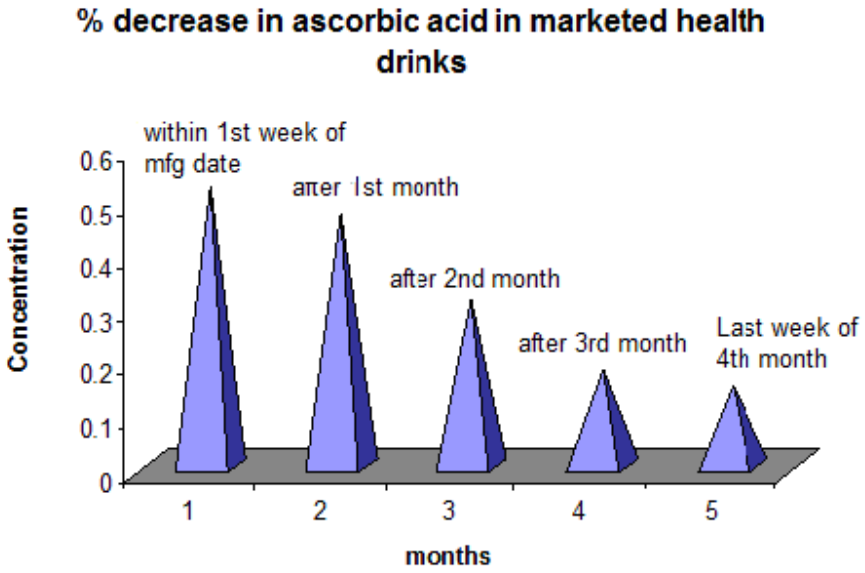
Fig 16. Chromatogram of ascorbic acid present in health drink
(Last week of forth month (before the expiry date)).

Marketed health drinks at various months	Concentration (µg/ml)
First week of manufacturing date	0.52
At the end of 1st month	0.47
At the end of 2nd month	0.31
At the end of 3rd month	0.18
Last week of fourth month (before the expiry date).	0.15

Table 6. Amount of ascorbic acid present in marketed health drinks at various month intervals

CONCLUSION

The ascorbic acid content was observed to be 0.52 µg/ml when estimated within first week from the date of manufacturing.



The ascorbic acid content was found to decrease

- by 1.10 fold after the first month.
- by 1.68 fold after the second month.
- by 2.89 fold after the third month.
- by 3.47 fold after the last week of fourth month.

when compared to that of quantity estimated in the product within one week from the date of manufacturing.

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