

## QUANTIFICATION OF ASCORBIC ACID IN SALAD COMPONENTS

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## ABSTRACT

The "Green salad" or "Garden salad" is most often composed of leafy vegetables such as lettuce varieties, spinach or rocket (arugula). Due to their low caloric density, green salads are a common diet food. The main ingredients of the green salad are Cabbage, Lemon and Tomato contain Vitamin C as a active constituent. Vitamin C (ascorbic acid, AA) is a water-soluble organic compound involved in many biological processes. A number of methods are available and are being developed for the extraction and characterization of Ascorbic acid from plants. This article deals with extraction and to develop a simple, efficient, reliable and cost-effective high performance liquid chromatographic (HPLC) method for the quantization of vitamin C in different components of the salads. The method is simple, rapid and has high specificity to ascorbic acid. Each analysis require no longer than 5 min. Qualitative estimation was carried out by thin layer chromatographic (TLC) method. HPLC separation was performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5 $\mu$ ) using Water(A) and 0.34% o-phosphoric acid (B) using an isocratic elution as follow: 0-30 min, 40%A-80% A, 60%B-20% B. The flow rate was 1.5 mL/min, column temperature 25°C, the injection volume was 25 $\mu$ l and UV detection was effected at 266 nm.

**Keywords:** Ascorbic acid, Salad, HPLC method.

## INTRODUCTION

Diet is believed to play an important role in four major diseases of advanced and transitional economies: cardiovascular disease, cancer, hypertension, and obesity. The degree to which diet is important in the prevention of these diseases is not known. Foods that contain components active in disease prevention and that aid specific bodily functions, in addition to being nutritious, are defined as functional foods<sup>1</sup>. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to be potential sources of medicinal substances<sup>2</sup>. Salad is any of a wide variety of dishes including vegetable salads; salads of pasta, legumes, eggs or grains; mixed salads incorporating meat, poultry, or seafood; and fruit salads<sup>3</sup> They may include a mixture of cold and hot, often including raw vegetables or fruits. Green salads include leaf lettuce and leafy vegetables with a sauce or dressing. Other salads are based on pasta, noodles, or gelatin. Most salads are traditionally served cold, although some, such as south German potato salad, are served warm. Green salads including leaf lettuces are generally served with a dressing, as well as various garnishes such as nuts or croutons. Salads may be served at any point during a meal<sup>4</sup>. They may be Appetizer salads, light salads to stimulate the appetite as the first course of the meal side salads, to accompany the main course as a side dish. Main course salads, usually contain a portion of protein. Palate-cleansing salads, to settle the stomach after the main course<sup>5</sup>. Tomato (*Lycopersicon esculentum* Mill.) is first main component of the salad. Tomatoes are the most popular vegetable in the home garden. They are available in a variety of sizes, shapes, and colors—including red, yellow, orange, and pink. Sizes vary from

the bite-sized cherry tomatoes to the giant beefsteak varieties. Tomatoes may be round, oblate (fruit are fattened at the top and bottom), or pear-shaped. Fleshy berry, globular to oblate in shape and 2-15 cm in diameter.

The immature fruit is green and hairy. Ripe fruits range from yellow, orange to red. It is usually round, smooth or furrowed. Tomatoes are low in calories and a good source of vitamin C and antioxidants<sup>6</sup>. Another cruciferous vegetable, cabbage is often overlooked by the modern homemakers but this fiber-rich vegetable is practically bursting with nutrients like vitamin C, which can reduce chances of developing certain cancers and help to prevent the spread of cancer once it has developed in the body. Cabbage also helps to stimulate the liver and supply a disease fighting cocktail of phytochemicals. Cabbage is a good detoxifier; it purifies blood and removes toxins (primarily uric acid which is a major cause for rheumatism, gout, arthritis, skin diseases, etc<sup>7</sup>). This detoxifying effect of cabbage is due to the presence of vitamin C and sulphur. Cabbage belongs to the family Crucifereae and its scientific name is *Brassica oleracea* L. It is used as a cooked vegetable or sometimes pickled or preserved by steaming and drying. The nutritional value is relatively high, although less than that of many other crops used in tropical areas as leafy vegetables. Green cabbage is a ball of thick, light green leaves<sup>8</sup>. The Citrus lemon contains important natural components, including citric acid, ascorbic acid, minerals and flavonoids<sup>9</sup> though health-related properties of citrus lemon have been associated with their content of vitamin C, but recently flavonoids have been also shown to play a role in this respect. Some researchers suggest that flavonoids have different biological functions, including antioxidative, anti-inflammatory, antiallergic, antiviral, antiproliferative, antimutagenic and anticarcinogenic activities<sup>10</sup>



Fig. 1: Green Salads

Vitamin C (chemical names: ascorbic acid and ascorbate) is a six-carbon Lactone and a valuable food component because of its antioxidant and therapeutic properties. It helps the body in forming connective tissues, bones, teeth, blood vessels and plays a major role as an antioxidant that forms part of the body defense system against reactive oxygen species and free radicals, thereby preventing tissue damage. It is widely used in the treatment of certain diseases such as scurvy, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility<sup>11</sup>. Vitamin C (ascorbic acid), which must be obtained from the diet, is an essential micronutrient required for normal metabolic functioning of the body. Therefore, a deficiency of this vitamin results in the symptoms of scurvy and death. This potentially fatal disease can be prevented with as little as 10 mg of vitamin C per day, an amount easily obtained through consumption of fresh fruits and vegetables. However, the current recommended dietary allowance (RDA) for vitamin C is set at 60 mg per day to provide an adequate margin of safety, as 60 mg/day would prevent the development of scurvy for about one month in a diet lacking vitamin C<sup>12</sup>. Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine and neurotransmitters. A deficiency in vitamin C results in a weakening of collagenous structures, causing tooth loss, joint pains, bone and connective tissue disorder and poor wound healing, all of which are characteristic of scurvy. Vitamin C is an important water-soluble antioxidant in biological fluids. It readily scavenges reactive oxygen and nitrogen species such as superoxide and hydroperoxyl radicals, aqueous peroxy radicals, singlet oxygen, ozone, peroxy nitrite, nitrogen dioxide, nitroxide radicals and hydrochlorous acid, thereby effectively protecting other biomolecules from oxidative damage<sup>13</sup>.

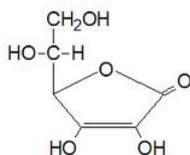


Fig. 2: Ascorbic acid

The main objective of the present study is to extract and develop a simple, efficient, reliable and cost-effective high performance liquid chromatographic (HPLC) method for the quantization of vitamin C in different components of the salads.

#### MATERIALS AND METHODS

**Plant material:**-Vegetables (Cabbages, Lemon and Tomato) were collected from Reliance fresh, Indrapuri Bhopal (M.P.).

#### Reagents and chemicals

The following chemicals and reagents were used:-

Ethanol, 1.0% w/w or w/v acetic acid, n-butanol, phenol, ammoniacal silver nitrate, O-phosphoric acid and water (HPLC grade) All chemicals were obtained from Shyam Brothers, 27- Sindhi market, Bhopal (M.P.).

#### Extraction of vitamin C

For vitamin C determination, vegetables were sliced, frozen into liquid nitrogen and stored at -80°C until the analyses were carried out. Frozen pulverized fruit samples were weighed (0.5 g for Lemon, 1.5 g for cabbage, 2 g for Tomato) and mixed with 2.5 ml of the extractant solution (3% O-Phosphoric acid(OPA) and 8% acetic acid for OPA-acetic acid extraction. The mixture was homogenized in a Politron PT 6000 high-speed blender at 18000 g (in ice and darkness) for 1 min and then centrifuged at 9000 rpm (refrigerated at 4°C) for 20 min. This procedure was repeated twice and the two resulting supernatants were mixed together. All extractions were carried out in quintuplicate.

#### Qualitative Profile (TLC & Paper Chromatography)

For Thin layer chromatographic studies of Ascorbic acid, precoated Silca gel F<sub>254</sub> aluminum plates ( 20 X 20cm) were used. The Ascorbic

acid was separated using ethanol: 1.0% acetic acid (9:1) as a mobile phase. The detection was carried out by UV 254nm.

For Paper Chromatography Whatmann filter paper was used as stationary phase. The Ascorbic acid was separated using two different solvents systems. Firstly n-butanol: acetic acid: H<sub>2</sub>O (4:1:5) taken as mobile phase. Second mobile phase Phenol: 1% acetic acid was taken. Detection was carried out by spraying with ammoniacal silver nitrate solution.

#### Preparation of Calibration curve

Calibration equation for AA was constructed by plotting the UV absorbance against the AA concentration at five concentration levels (analyzed in triplicate). UV absorbance (y) of AA over a concentration (x) range of 05-30 ppm was linear  $y = 0.0253 x$  with a regression coefficient( $r^2$ ) of 0.9905 (Fig: 3).

#### Quantitative estimation of Ascorbic acid by HPLC

The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrance, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 μm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of Water (A) and o-phosphoric acid (B) using an isocratic elution as follow: 0–10 min, 90% A–99% A, & 0.1 %B–0.2% B. The flow rate was 1.5 mL/min, and a column temperature of 25°C. The injection volume was 25μl, and UV detection was effected at 266 nm. After extraction, the ascorbic acid (10μg/ml) was subjected to HPLC column and the obtained records were superimposed on the retention time values of the standard ascorbic acid.

#### RESULT AND DISCUSSION

Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidized to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. The extracted sample was subjected for qualitative estimation by TLC analysis (Table 1) and Paper chromatography (Table 2). UV scanning of different samples was carried out at 200-400nm (Fig:2). Calibration equation for AA was constructed by plotting the UV absorbance against the AA concentration at five concentration levels (analyzed in triplicate). UV absorbance (y) of AA over a concentration (x) range of 05-30 ppm was linear  $y = 0.0253 x$  with a regression coefficient ( $r^2$ ) of 0.9905 (Fig: 3).The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. HPLC separations of isolated samples with reference to standard were performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5μ). Thus, chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines<sup>14</sup>. Good results were obtained using a mixture of water and orthophosphoric acid as the mobile phase. The concentration of orthophosphoric acid was studied over the range 0-0.5% and a concentration of 0.2% was found to be optimal. The flow-rate significantly influenced AA retention time; the best flow rate was 1.0 ml/min (optimized between 0.4 and 1.6 ml/min) due to the better retention time ( $4 \pm 0.06$  min) and resolution for AA and other compounds. The retention time of different components of salad was tabulated in table 3 and figure 4.

#### CONCLUSION

The 3% O-Phosphoric acid (OPA) and 8% acetic acid method affords enough sensitivity and selectivity in L-ascorbic acid determination in vegetable components of salad. The quantification of Ascorbic acid was carried out by HPLC. The present work revealed that this developed method was simple, efficient, reliable and cost-effective for the quantization of vitamin C in different components of the salads. The method is simple, rapid and has high specificity to Ascorbic acid.

Table 1: TLC Profile of Ascorbic acid

S.No.	Sample	Solvent System	Detection	R <sub>f</sub> of Sample	Inference
1.	Std ascorbic acid	ethanol: 1.0% acetic acid (9:1)	UV 254nm	0.51 (Blue spot)	Ascorbic acid
2.	Vit.C(Tomato)	ethanol: 1.0% acetic acid (9:1)	UV 254nm	0.50 (Blue spot)	Ascorbic acid
3.	Vit.C(Cabbage)	ethanol: 1.0% acetic acid (9:1)	UV 254nm	0.74 (Blue spot)	dehydroascorbic acid
4.	Vit.C(Lemon)	ethanol: 1.0% acetic acid (9:1)	UV 254nm	0.55 (Blue spot)	isoascorbic acid

Table 2: Paper chromatographic Profile of Ascorbic acid

S.No.	Sample	Solvent System		Detection	R <sub>f</sub> value of Sample		Inference
		Mobile Phase A	Mobile Phase B		A	B	
1.	Std ascorbic acid	n-butanol:Acetic acid: H <sub>2</sub> O(4:1:5)	Phenol-1% acetic acid	Sprayed with Ammoniacal AgNO <sub>3</sub>	0.37	0.36	Ascorbic acid
2.	Vit.C(Tomato)	....."	....."	....."	0.4	0.39	isoascorbic acid
3.	Vit.C(Cabbage)	....."	....."	....."	Tailing	0.4	dehydroascorbic acid
4.	Vit.C(Lemon)	....."	....."	....."	0.38	0.34	Ascorbic acid

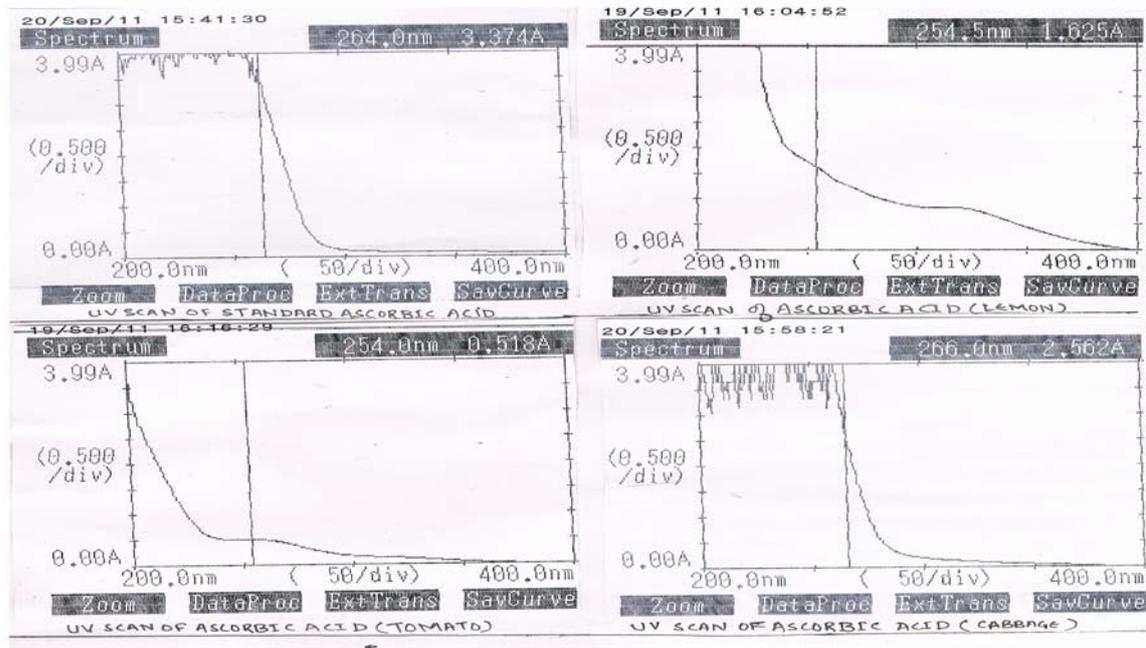


Fig. 2: UV scanning of Standard and Samples

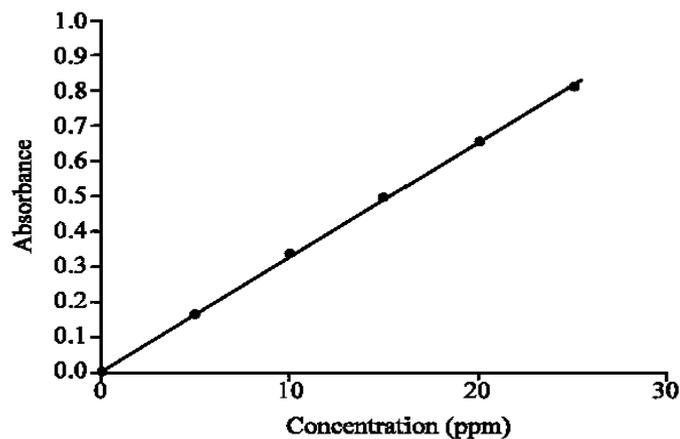


Fig. 3: Standard Curve of ascorbic acid

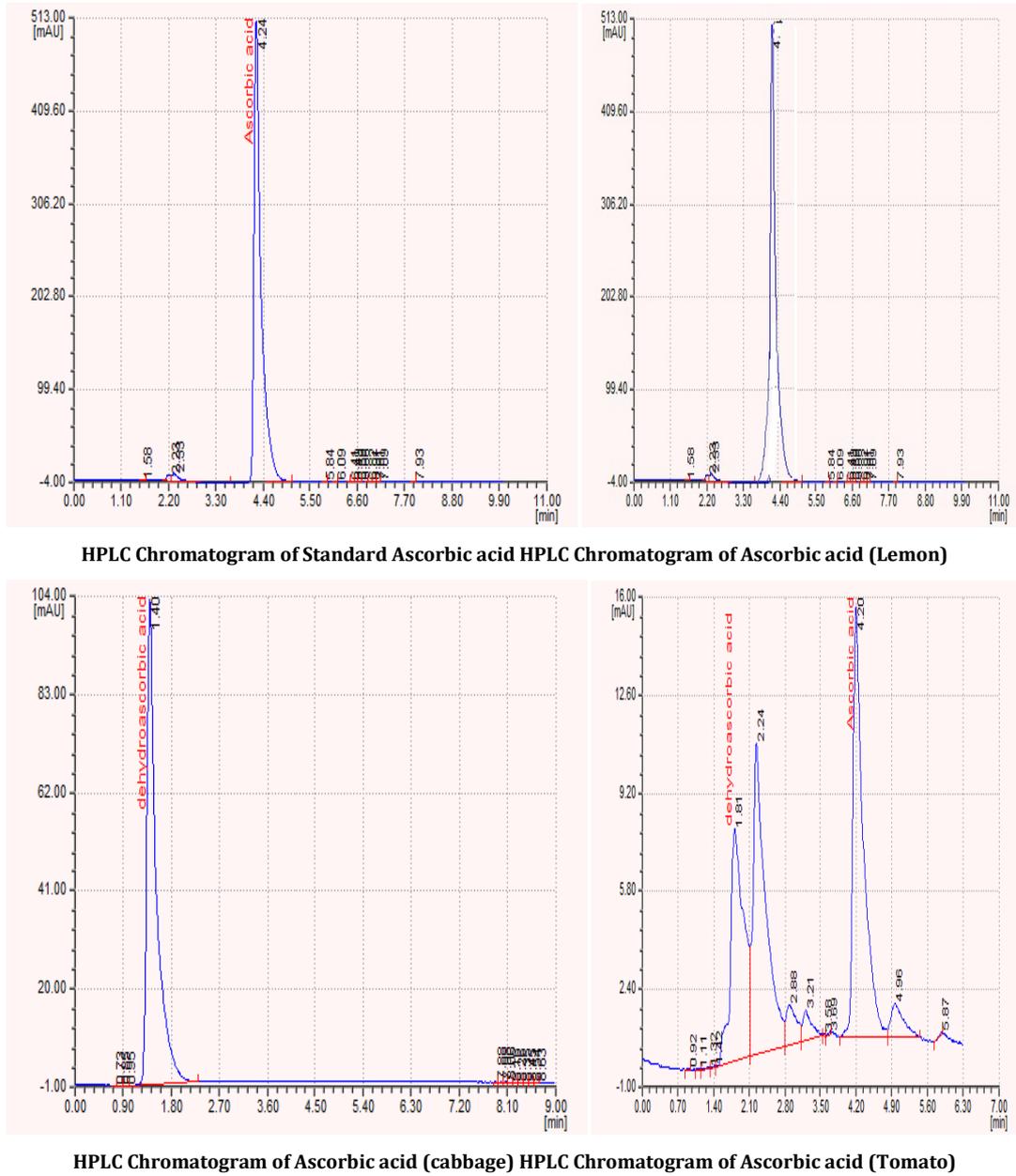


Fig. 4: HPLC Chromatogram of Ascorbic Acid

Table 3: HPLC Analysis of Ascorbic acid

S. No	Sample	Height	Area	Conc.	RT	Inference
1.	Standard Ascorbic acid	51422	582540.3	97.7197	4.24	Ascorbic acid
2.	Ascorbic acid (lemon)	56711	23499.3	98.7015	4.00	Ascorbic acid
3.	Ascorbic acid (cabbage)	10361	127527.7	99.8387	1.80	Dehydroascorbic acid
4.	Ascorbic acid (Tomato)	1494	21116.6	35.4635	4.20	Ascorbic acid
		808	14718.0	24.7177	1.81	Dehydroascorbic acid

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