



VITAMIN: ANALYSIS OF VITAMIN A, B AND C ABSTRACT –

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ABSTRACT

This paper describes the analytical method for residual Oxaliplatin testing on manufacturing equipment and is used in cleaning verification of manufacturing equipment to ensure a high degree assurance. Proposed method is proven to be sensitive, simple and accurate for quantification of residual Oxaliplatin. Retention time of Oxaliplatin was found to be 8.903 with isocratic flow rate of 0.8 mL/min at detection wave length of 210nm with 40°C column oven temperature, LOD, LOQ concentrations of Oxaliplatin was found to be 0.06ppm and 0.12ppm respectively.

KEYWORDS: Oxaliplatin; Residual; Cleaning Validation; Swab sampling, Manufacturing Equipment.

INTRODUCTION

Vitamins

Vitamins enable your body to develop and create and likewise assume critical parts in substantial capacities, for example, digestion, resistance and processing. There are 13 basic vitamins, including vitamins A, C, D, E and K and B vitamins, for example, riboflavin and folate.

Vitamins are micronutrients that are fundamental to life, and a considerable lot of them assume an imperative part in direction of cerebrum working.^[16]

There are two sorts of vitamins – water solvent and fat dissolvable vitamins. Water solvent vitamins incorporate vitamin B, C. Fat solvent vitamins incorporate vitamin A, D, E and K.

Analysis of vitamins --Vitamins can be analysed by many methods. The selection of method depends upon which vitamin is been analysed.

They can be analysed directly or either indirectly

→The direct analysis methods – HPLC, Immunoassay, Colorimetry, Fluorimetry, Chemiluminescence, Amperometry Etc.

→Indirect analysis methods – Metabolite Loading Test, Erythrocyte Fragility Test, Prothrombin Time Test Etc.^[16]

Vitamin-C

Vitamin C, otherwise called ascorbic corrosive and L-ascorbic corrosive, is a vitamin found in sustenance and utilized as a dietary supplement. The malady scurvy is averted and treated with vitamin C containing

sustenance's or dietary supplements. It might be taken by mouth or by infusion. Vitamin C was found in 1912, separated in 1928 and first made in 1933, making it the principal vitamin to be manufactured.^[10] It is on the World Health Organization's List of Essential Medicines.^[16]

Sources – Orange, Kiwi, Cauliflower, Kale, Pine Apple, Broccoli, Papaya, Red Ringer Pepper, Strawberry, Guava, Grape Organic Product, Potato, Radish, Growers, Melon, And So On.

Vitamin C Analysis Methods

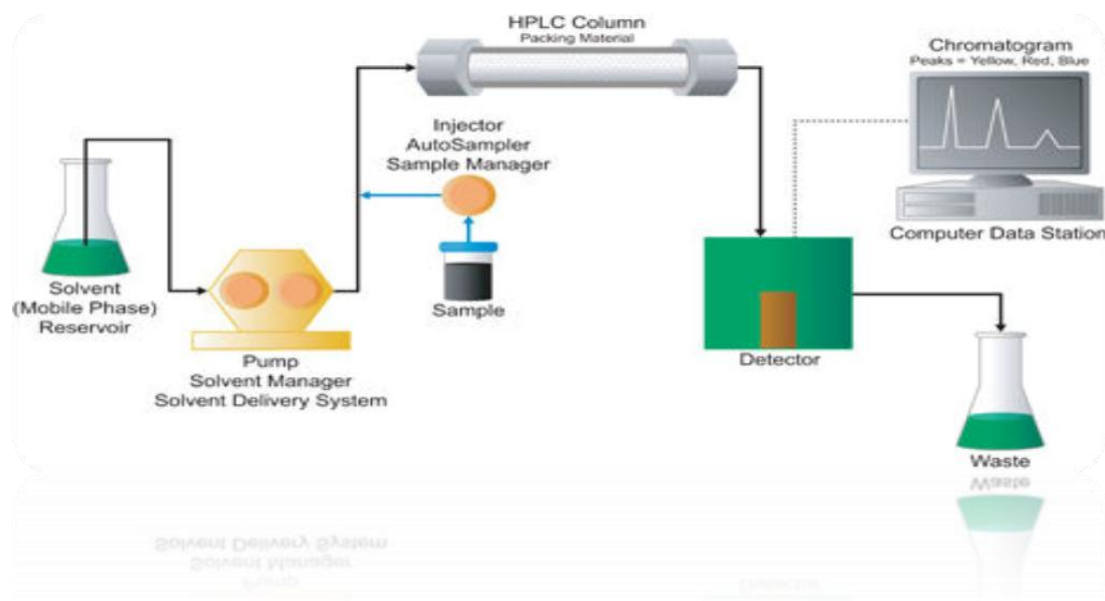
High Performance Liquid Chromatography (Hplc)

This is an official method for the analysis of Vitamin C. Also used to determine iso-forms of the vitamin, reported by laxmansawant*, balaprabhakkar and Nancy Pandita in Journal of Analytical & Bioanalytical Techniques – O under the title quantitative HPLC Analysis of Ascorbic Acid and Gallic Acid in phyllanthusemblica.

A reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous estimation of ascorbic and gallic acid in Phyllanthusemblica L. (Euphorbiaceae). A C18 section was utilized with a slope elution of methanol and 0.1% (v/v) acidic corrosive in HPLC-review water as versatile stage at a stream rate of 0.9 ml min⁻¹. UV detection was performed at 278 nm. The strategy was approved for exactness, accuracy, linearity, specificity and affectability as per International Conference on Harmonization rules. Measures of ascorbic and gallic corrosive distinguished in solidified dried concentrate of the plant were broke down. Add up to run time was 50

min. Ascorbic and gallic corrosive was eluted with maintenance times of 3.60 and 10.77 min separately. Adjustment plots were straight finished the focus ranges 30– 90 µg ml⁻¹ for ascorbic corrosive and 5– 15 µg ml⁻¹ for gallic corrosive, individually. Cutoff points of

discovery were 1.42 and 1.56 µg ml⁻¹ and breaking points of evaluation were 4.32 and 4.73 µg ml⁻¹ for ascorbic and gallic corrosive, individually. Recovery was 99.37% and 98.68% for ascorbic and gallic corrosive, individually.^{[3][4]}



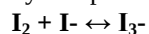
2) Titrimetric Analysis of Vitamin C

Following is an official analytical procedure from bureau of Indian standards and also as per Indian Pharmacopeia 1985.^[5]

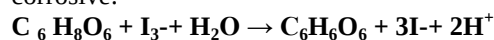
Principle

One approach to decide the measure of vitamin C in sustenance is to utilize a redox titration. The redox response is superior to a corrosive base titration since there are extra acids in a juice, however few of them meddle with the oxidation of ascorbic corrosive by iodine.^[5]

Iodine is moderately insoluble, yet this can be enhanced by complexing the iodine with iodide to shape triiodide:



Triiodide oxidizes vitamin C to shape dehydroascorbic corrosive:



For whatever length of time that vitamin C is available in the arrangement, the triiodide is changed over to the iodide particle rapidly. Be that as it may, when the all the vitamin C is oxidized, iodine and triiodide will be available, which respond with starch to shape a blue-dark complex. The blue-dark shading is the endpoint of the titration.

This titration technique is fitting for testing the measure of vitamin C in vitamin C tablets, juices, and new, solidified, or bundled foods grown from the ground. The titration can be performed utilizing just iodine arrangement and not iodate, but rather the iodate

arrangement is more steady and gives a more precise result.^[5]

Procedure

Weigh required measure of strong iodine in a receptacle, include strong potassium iodide, at that point include refined water and break up iodine and potassium in it. Transfer it into a clean 100ml volumetric flask and make up 100ml volume with refined water. Now a starch arrangement is set up by including starch in refined water and make up the volume with the same. The organic product juices are extricated and separated utilizing muslin material. The iodine sol of 0.01M in burette and take the organic product squeeze in a measuring glass and include starch arrangement and titrate it with iodine until the point when the shading change from shade of natural product juice to blue-dark shading, simultaneous readings are taken.^[5]

CONCLUSION

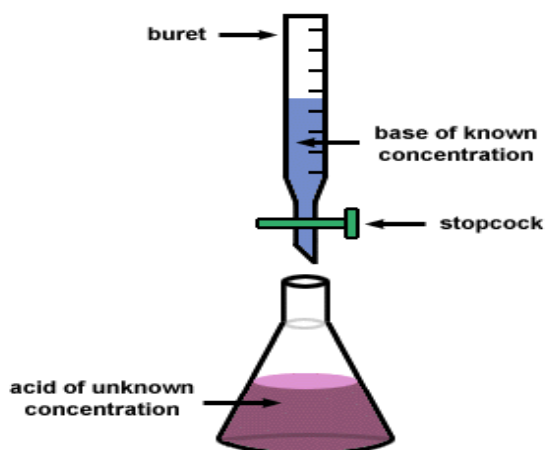
The accompanying are level of ascorbic corrosive in various organic product juices

Lemon juice-31.45%

Squeezed orange 49.66%

Citrus maxima juice – 70.4%

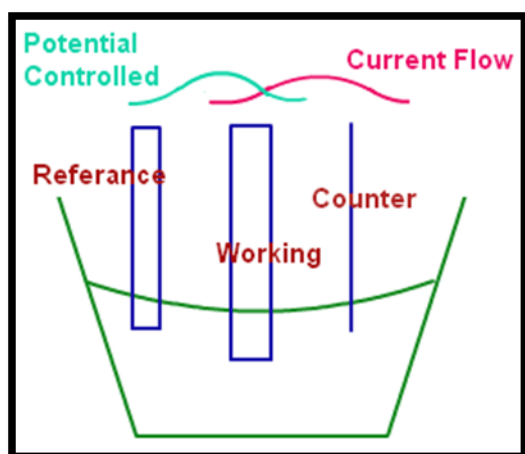
The level of ascorbic corrosive was observed to be less in lemon juice and more in citrus maxima juice. Here the starch arrangement is added before the titration because of which some of organic product content responded starch and perpetual complex was shaped which couldn't be oxidized.



3) Amperometric Assays

An oxygen anode, secured by a thin, dynamic layer of artificially insolubilized ascorbate oxidase especially distinguishes by estimating oxygen take-up of ascorbic corrosive in phosphate cradle. The record of current response to substrate happens for 1-2 minutes. The ascorbic destructive esteems chose in various examples of common item squeezes are in awesome simultaneousness with the data obtained by titration and polarography.^{[6][9]}

Perfect reaction conditions of vitamin C affirmation and the potential results of block of various blends are in like manner broke down. Of the 35 phenols, fragrant amines and acids attempted chlorogenic destructive simply can cause a positive bungle gave that the impetus layer has been set up from ascorbate oxidase of high virtue.



4) Effect of Ascorbic Acid on Osmotic Fragility of Normal Erythrocytes in Saline

Principle

Vitamin C (ascorbic corrosive) can hinder in vitro, the hemolysis in sheep red cells because of supplement and saponin. Around then it was gathered, that the Vitamin C may have acted some place on the red cells adjusting their protection from the hemolytic operator. Since in these trials it was impractical to discount the likelihood

of any obstruction of the Vitamin C with the hemolytic specialists, diverse arrangements of ordinary saline were utilized as the hemolytic operator for this situation. Keeping in mind the end goal to counteract additionally any obstruction of the Vitamin C with any constituent of ordinary plasma, a suspension-of red cells in saline was utilized. Thusly, any impact on the hemolytic framework must be thought to be because of direct activity of the Vitamin C on the red cells. The strategy utilized was like that utilized on the delicacy trial of the red cells with some modifications.^[9]

Procedure

- 1) First, new citrated blood of solid people was centrifuged and washed four times with typical saline.
- 2) Then a suspension with one a player in red cells and two sections of ordinary saline was made.
- 3) In four arrangement of ten tubes each were put ordinary saline, refined water and Vitamin C.

To every one of the containers of every arrangement a drop of the washed red cells suspension was included. Every one of the tubes were then put in a hatchery at 37 C. Furthermore, saw following 1, 12 and 24 hours. The analysis was rehashed with erythrocytes from two extra solid people. For all intents and purposes indistinguishable outcomes were acquired. These outcomes demonstrate plainly that the vitamin C shields the red cells from the hemolytic activity of hypotonic arrangements of saline. Since the hemolytic framework is made just of refined water and salt at various weakenings, it can likewise be sensibly expected that the Vitamin C must follow up on the red cells expanding their protection, despite the fact that the system of this activity is extremely hard to clarify with our knowledge.^{[7][8]}

Spectrophotometric Analysis of Vitamin C

Principle

Ascorbic acid displays an absorbance maxima at 265micrometer in neutral aqueous solution. The maximum is shifted to 245micrometer by traces of mineral acids. Copper ions greatly effect stability of ascorbic acid. It has been shown that this effect can be eliminated by addition of KCN to the water used as a solvent for the spectrophotometric determination.

Procedure

Method of estimation of ascorbic acid in the presence of absorbing impurities are based on absorbance reading being taken before and after determination of ascorbic acid thus yielding blank.

In one procedure the spectrum id determined in a solution containing KCN. The solution is then subjected to prolong UV irradiation until the absorbance attains a constant value. The difference between the readings is proportional to ascorbic acid. The another method the ascorbic acid is destroyed by oxidation in the precursor

of Cu^{2+} , to dihydroascorbic acid which absorbs negligibly.^{[9][10]}

Vitamin –B

B vitamins are a class of water-soluble vitamins that assume imperative parts in cell digestion. In spite of the fact that these vitamins share comparative names, look into demonstrates that they are chemically distinct vitamins that regularly exist together in similar substances. Singular B vitamin supplements are alluded to by the particular number or name of every vitamin: B1 = thiamine, B2 = riboflavin, B3 = niacin and so forth. Some are preferable known by name over number: niacin, pantothenic corrosive, biotin and folate.^[16]

Every B vitamin is either a cofactor (by and large a coenzyme) for key metabolic procedures or is an antecedent expected to make one.

Sources – Meat, Angle, Carrot, Papaya, Egg, Cheddar, Beans, Green Peas, Lentils, Sweet Potato, Mango, Broccoli, Peach, Avocado, Pepper, Yeast, Liver, Grains, Drain, Yogurt, Meat, Soymilk, Shellfish and so forth.

Vitamin B Analysis Methods

1) Determination of Selected Vitamins B in Fruit and Herbal Teas(HPLC)

The target of this examination was to decide the levels of vitamins B2, B6 and B12 in seven unique composites of the soil teas. The chromatographic division was performed in a C18 section with isocratic elution and UV discovery at 280 nm. The examinations were made by standard expansion strategy. Every single considered vitamin were isolated underneath 10 minute.

- HPLC is the favored procedure for vitamin division, on account of its high selectivity. The synchronous assurance of a few water-solvent vitamins is troublesome and regularly a wide range of examinations must be performed.

- In this, the strategy for synchronous assurance of three vitamins: B1, B2, B6 by HPLC with UV-Vis discovery is accounted for. The proposed strategy is straightforward and quick and it is conceivable to recognize and at the same time decide examined vitamins in under 10 min with just a single injection.^[12]

2) Immunoassay of Vitamin Analysis

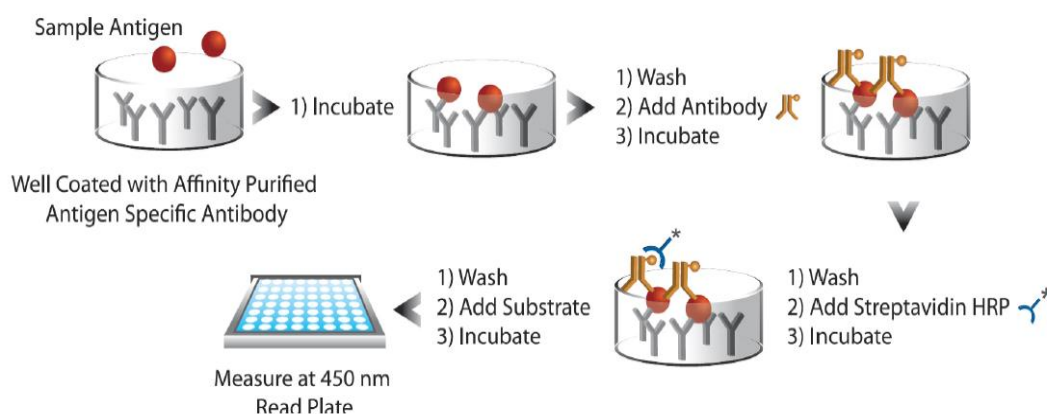
There are a few kinds of immunoassays, for example,

- 1) Enzyme Linked Immuno-Sorbent Assay that uses antibodies connected to catalyst.
- 2) Radio-immunoassays rather use a radio-isotope and because of radioactive transfer concerns these are presently out dated.
- 3) Fluorescent immunoassays use antibodies marked with fluorophore however need affectability when contrasted with past two.

Vitamin-B12 can be examined by Immunoassay. These measures however rely on accessibility of monoclonal antibodies that decide their specificity and affectability.

Competitive Immunoassay for Examination of Vitamin B(12)

Coordinate focused chemical connected immunosorbent examine (ELISA) was delivered for derivatized vitamin B(12) by making chicken egg yolk immunoglobulins (igy) against derivatized vitamin B(12) and refined using preferring chromatography. Checkerboard test was performed with vitamin B(12) neutralizing specialist and vitamin B(12)- dissolvable phosphatase conjugate took after by its conjugate depiction using splendid (UV) spectroscopy and predominant liquid chromatography (HPLC). The analyze was endorsed with the HPLC system in regards to affectability, specificity, precision, and recovery of vitamin B (12).^{[9][13]}



3) Fluorimetric Analysis

Principle

There are 3 general procedures used in fluorimetric determination of riboflavin

1. Direct determination
2. Direct additive determination
3. Adsorption additive determination

One of the commonly used procedure is the direct determination.

The direct determination is used for mixtures which are free of interfering pigments or other interfering substances and contain relatively high concentration of riboflavin.

The direct additive determination – many materials such as poultry and live stock feed mixtures, pharmaceuticals and grain products may contain other fluorescent compounds and pigments which may interfere in direct determination procedure. In such cases, direct additive adsorption methods are used. In this addition of known quantity of riboflavin to the assay solution is used to compensate for interference substance which may absorb the incident light.

Adsorption additive determination – in this procedure most interference substance are eliminated by 6 an adsorption step and those not so eliminated are compensated for by means of addition of known quantity of riboflavin.

Procedure - Direct determination

The weight of the sample to be used depends on its riboflavin content. The solubility of riboflavin in water, at room temperature is approximately 100mg/L.

An appropriate quantity of sample is weighed and transferred to volumetric flask. Add hot distilled water and agitate for few minute. Some samples of riboflavin may require additional heating. The solution is cooled to room temperature, then dilute it with distilled water and mix well. It is centrifuged and/or filtered of necessary. The crystal clear solution is needed for fluorescence measurement. The filtered solution is diluted with distilled water to yield concentration of about 0.2mcg of riboflavin per ml.

The sample solution is placed in cuvette and the readings are taken. (science riboflavin is destroyed by UV light, the reading is not stable and must be taken as rapidly as possible).

The blank determination is conducted and then compared. The fluorescence in the above test should not be greater than mentioned for standard solution treated for same manner. This gives indication of purity.^[11]

Spectrophotometric analysis

Riboflavin has characteristic uv absorption spectrum in water with maxima at 267micrometr. This property provides basis for analysis of riboflavin. The method is based on the assumption that extraction with chloroform will remove interfering impurities from an aqueous solution.

Procedure

Weighed quantity of riboflavin is taken in volumetric flask. To it add water and sodium hydroxide solution. Shake gently until solution is complete then acetic acid is added and diluted to 1000ml with water and mixed. 20ml of aliquote is transferred to sepreting funnel and shaken for 1min with 25ml of chloroform. After separation the chloroform layer is discerded. The extraction is repeated with 25ml portion of chloroform. This layer is again discerded.

The absorbans of the clear aqueous layer is determined in b1cm quarts cell at 267micrometer. Water is used as reference liquid. The procedure is repeated on a standard reference sample of riboflavin.^[11]

Silicotungstic acid method method

Principle

Thiamine in tablet and in solution may be determined by precipitation with silicotungstic acid. This method is official in b p (2,11)

Procedure

The sample is dissolved in water, acidified with hcl and heated to boiling a solution of silicotungstic acid is added drop by drop to the hcl, then with water and finally with acetone. The residue is dried to contant weighed. Each gram is equivalent to 0.1986g of thiamine hydrochloride.^[11]

Vitamin A

Vitamin A will be a gathering of unsaturated dietary natural aggravates that incorporates retinol, retinal, retinoic corrosive and a few provitamin A carotenoids (most prominently beta-carotene).^[16]

Vitamin A has various capacities:- It is vital for development and improvement,

- For the upkeep of the safe framework

- And great vision.

Vitamin An is required by the retina of the eye as retinal, which consolidates with protein opsin to shape rhodopsin, the light-retaining particle vital for both low-light (scotopic vision) and shading vision.

Sources - Vitamin An is found in numerous foods:cod liver oil, liver, Ghee, capsicum, red, sweet potato carrot, broccoli, margarine, kale, collard greens, Butternut squash, dandelion greens, spinach, pumpkin, collard greens, cheddar, melon melon, egg, apricot, papaya, tomatoes, mango, pea, broccoli, drain, ringer pepper.^[16]

Vitamin A Analysis Methods

1. HPLC

Vitamin A (Retinol) is controlled by superior fluid chromatography with UV-discovery after saponification and extraction. The technique is specifically usable for tablets containing a lot of betacaroten and for items containing a little measure of vitamin A. Least

centralization of the example: About 0.3 mg of vitamin A/g.

Procedure

- 1) Pulverise 20 tablets in a crushing factory. Weigh out precisely the powder.
- 2) Add 10 ml of sodium ascorbate arrangement (2%) and warm in a steam shower for 5 minutes with much of the time shaking
- 3) Add 30 ml of alcoholic BTH-arrangement (0.1%) and 3 ml of potassium hydroxide (17M).
- 4) Connect the flagon of an air condenser and reflux for 30 minutes on a steam shower (shake as often as possible).
- 5) Cool and exchange with 30 ml of sodium sulfate 3% and 100 ml of ether to a 500 ml isolating pipe containing 100 ml of ether. Shake for 2 minutes.
- 6) Let remain until the point that the layers are obviously isolated (around 30 minutes), and release the lower watery layer (if an emulsion is shaped, include a few drops of ethanol 99%). Wash the ether extricate with 4 x 50 ml of water; shake precisely at the outset so as to maintain a strategic distance from emulsification.
- 7) Afterwards pour a few ml of water stage into an axis tube containing a couple of drops of phenolphthalein-R1. In the event that it is red - keep washing until the point when the washings are never again hued red. A while later, exchange the ether layer to a 250 ml volumetric jar or a round-base jar.
- 8) Tablets and arrangements - A+B: Transfer through a cotton plug secured with anhydrous sodium sulfate by ether to a round-base cup. Vanish in vacuum and weaken with n-heptane until a last grouping of 3-4 mcg/ml is found. C: Transfer to a 250 ml volumetric jar and top off to volume with ether. 20.00 ml in a 50 ml jar is dissipated under a steam of nitrogen until the point that a rest of 2 ml is cleared out. Weaken with n-heptane to a last volume of 50 ml.
- 9) Transfer example and standard to vials and infuse 100 ml of the arrangements (twofold). Record the region or stature of the A-vitamin peaks.^[18]

2. Spectrophotometric Analysis

Principle

This method is based on UV absorption characteristics of vitamin as per USP XV procedure, the sample is vsaponified, the vitamin A is extracted with ether, the ether evaporated and the vitamin A is dissolved in isopropanol. The absorbance of the solution is measure at the absorbtion maxima and at 2 "fixation points", one to the right and the other to the left of the maxima. The absorbance maxima is correcter for irrelevant absorbance by the application of correction formula.

Procedure

SAPONIFICATION - An accurately measured amount of the sample, containing vitamin A not more than 1g of fat is transferred to saponification flask. This is boiled under the reflux condenser, with alcohol and KOH solution for 30min. the solution is cooled and water is

added. The resulting solution is transferred to separator and extracted with 4 successive portions of ether. The extracts are combined and washed with water. The washing is repeated until washing gives no color. The washed ether is transferred to volumetric flask and diluted.

DETERMINATION – aliquote of ether solution of unsaponifiable extract is evaporated to about half of its volum. Without the application of heat. The remaining ether is removed in a stream of nitrogen or by vacume. The recedue is dissolved in sufficient isopropyl alcohol to give an expected concentration of vitamin A in each ml. Such that it will give an absorbance at 325 micrometer. The the wavelengths at which the absorbance is checked are 310, 325, 334 micrometer.^{[10][11]}



Colorimetric Method (Carr-Price Method)

In USP XIV the colorimetric assay was used as an identification test, in which the blue color was quantitatively standardized against vitamin A reference standard.

Principle

Anhydrous antimony trichloride in chloroform reacts with the dilute solution of vitamin A to form transient blue color. (which is basis of Carr price method).

The reaction takes place between anhydrous antimony trichloride and unsaturated side chain of vitamin A. The carotenes, polyene acids and various other materials present in natural oils produce the same color. T5he color rapidly reaches a maximum intensity and just as rapidly fades away. The reading should be made between 10-15min after the sample has been mixed with the reagent.

Procedure

First vitamin A is extracted and weighed quantity is taken into a beaker. To the above beaker add required amount of water to make the solution dilute. to the above dilute solution add chloroform and mix well. The add

antimony trichloride which reacts with the sample and gives the blue color. then place this solution in colorimeter and note the reading. add the correction factor if needed. The reading should be made between 10-15min after the sample has been mixed with the reagent.^{[10][11]}

CONCLUSION

Vitamins such as A, B and C play a crucial role in the regulation of different physiological functions of body. They also serve as an important dietary supplements. over the decade the market of methods have been reported for the Analysis of vitamins. These completely developed analytical methods over the decade include HPLC, colorimetric analysis, amperometric analysis and spectrophotometric analysis. They either focus on the assay and qualitative evaluation of vitamins or evaluate the biological activity of vitamins. Other tests that determine the functioning capacity include prothrombin test, erythrocyte enzyme activation test, immunoassay, fluorimetry, chemiluminescence and helpful in providing intrinsic knowledge regarding the behavior of vitamins in human body which can only be provided by these techniques. There are some methods which help us in qualitative and quantitative estimation of vitamins in our body such as electroluminescence, inductive coupled plasma (ICP) mass spectrophotometry, atomic absorption spectroscopy etc.

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