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# STUDY OF IODATE- IODIDE MIXTURE IN VITAMIN C AND ITS ANALYTICAL APPLICATIONS IN PHARMACEUTICALS

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# ABSTRACT

A simple spectrophotometric method is developed for the determination of vitamin C in pharmaceutical formulations. The method is based on the reaction of vitamin C with a mixture of potassium iodate (KIO<sub>3</sub>) and potassium iodide (KI) to form yellow coloured product in aqueous medium at room temperature  $(25 \pm 1^{\circ}C)$ . The reaction is followed spectrophotometrically by measuring absorbance at 352 nm. Under optimized experimental conditions, Beer's law is obeyed in the concentration range of 1.0-3.5 µg/ml. The method is validated with respect to accuracy, precision, limits of detection and quantitation. Robustness testing is also conducted to evaluate the effect of minor changes to the absorbance of the product.

# **KEYWORDS**

Vitamin C, Spectrophotometry and Robustness.

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#### **INTRODUCTION**

Ascorbic acid is a naturally occurring organic compound (Figure No.1). It is widely distributed in nature and obtained from citrus fruits and many vegetables. The pure form is a white crystalline powder. It dissolves in water to give mildly acidic solutions<sup>1</sup>. It is an essential nutrient in human diets and necessary to maintain connective tissue and bone. It's biologically active form functions as a reducing agent and coenzyme in several metabolic pathways and considered as an antioxidant. Ascorbic acid is one form "vitamer" of vitamin C.

Chemically; it exists in L-ascorbic acid form which does not occur in nature. It may be synthesized artificially<sup>2</sup>. The IUPAC name of ascorbic acid is (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H furan -5-one and commonly known as vitamin C. Vitamin C have various major biologically activities such as maintenance of the organism, prevention of vitamin C deficiency (scurvy), promotion of collagen biosynthesis, inhibition of melanogenesis and antioxidation<sup>3-8</sup> etc. Commercially it is available in various formulations such as tablets, injections, syrups and capsules etc. Assay is an important method for checking the commercially formulated products. Vitamin C has also long been recognized as an important nutrient in several food products. The reduced form of the vitamin C is referred to as L-ascorbic acid, and the oxidized form is referred to as dehydroascorbic acid. In humans, both forms are biologically active. The total vitamin C activity is the sum of both forms.

Vitamin C is added during the manufacture of juices or soft drinks to improve their nutritional value or to prevent the autoxidation of commercial products. Owing to the wide use of L-ascorbic acid in canned fruits, vegetables and drugs, numerous analytical methods have been developed for the determination L-ascorbic acid, includes titrimetry<sup>9</sup>, of spectrophotometry<sup>11–14</sup>, fluorimetry<sup>10</sup> gas chromatography<sup>15</sup>. polarography<sup>16</sup>, flow injection, conductometry<sup>17</sup> high-performance liquid and  $(\text{HPLC})^{18-24}$ . The chromatography developed methods are sensitive but require lengthy procedures and difficult reaction condition. Some of them involves costly and carcinogenic reagent and suffering from serious interferences.

The aim of present investigation is to demonstrate a simple, sensitive, fast, simple and economical method for the determination of vitamin C in pure and dosage forms. In this method, the iodate reacts with iodide to form iodine which is relatively insoluble, but this can be improved by complexing the iodine with excess iodide *in-situ* to form triiodide. Triiodide oxidizes vitamin C to form dehydroascorbic acid and re-forms iodide in the process.

# EXPERIMENTAL

#### Apparatus

Spectral runs were made on Spectronic 200 Visible Spectrophotometer (Thermo Scientific, USA) Standard sample compartment accommodates both 10 mm square cuvettes and test tubes up to 25 mm (1 inch) diameter.

#### **Materials and Reagents**

- Vitamin C was purchased from Sigma Aldrich, USA and used as working standard.
- Pharmaceutical formulations of vitamin C such as Ca – C 1000 Sandoz (Novartis Consumer Health, Switzerland), Redoxon (Bayer Consumer Care Ltd, Switzerland) and Cal–C – Vita (Bayer Consumer Care Ltd, Switzerland) were purchased from local market.
- Potassium Iodate (KIO<sub>3</sub>) was purchased from Sigma Aldrich, USA.
- Potassium Iodide (KI) was purchased from Sigma Aldrich, USA.

#### Solution Preparation

#### Standard vitamin C solution

A stock solution of vitamin C (100  $\mu$ g/ml) was prepared by dissolving 25 mg vitamin C in 250 ml volumetric flasks with distilled water. The stock solution was used to prepare the working solutions by suitable dilutions with distilled water. The solutions were stable at least 10 days at room temperature (25 ± 1° C).

#### **Procedure for determination of vitamin C**

Into a series of 10 ml volumetric flasks, different volumes (0.1-0.35) ml of standard vitamin C (100  $\mu$ g/ml) solution corresponding to 10-35  $\mu$ g were pipetted. To each flask, 1.0 ml KIO<sub>3</sub> (1000 µg/ml) and 2.0 ml KI (2500 µg/ml) were added and diluted to volume with distilled water. The reaction was allowed to proceed at room temperature  $(25 \pm 1^{\circ} \text{ C})$ and absorbance was measured at 352 nm against the reagent blank prepared simultaneously (Figure No.2). The calibration curve was constructed by plotting the absorbance against the initial concentration of vitamin C. The content of vitamin C is calculated either from the calibration curve or corresponding regression equation.

#### Procedure for determination of vitamin C in dosage forms

One tablet (claiming 1 g of vitamin C) was accurately weighed and finely powdered. A quantity of the powder equivalent to 25 mg of vitamin C was extracted by shaking with 100 ml of distilled water, followed by another two extractions each with 50 ml distilled water. After passing through a 0.45 µm millipore filter, the solution was diluted with distilled water to obtain a concentration of 100 µg/ml. It was further diluted according to the need and then analyzed following the proposed procedure. The nominal content of the tablet was calculated either from the previously plotted calibration graphs or using regression equation.

#### Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) is the point at which a measured value is longer than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessary quantified. The limit of quantitation (LOQ) is the lowest concentration or amount of analyte that can be determined quantitatively with an acceptable level of repeatability precision and trueness. According to International Conference on Harmonization (ICH) guidelines the following expressions are used to evaluate LOD and LOQ.

 $LOD = 3.3 \times S_0 / b$  and  $LOQ = 10 \times S_0 / b$ 

where  $S_0$  and b are standard deviation and slope of the calibration line, respectively.

#### **Method Validation**

Method validation is closely related to method development. When a new method is being developed, some parameters are already being evaluated during the 'development stage' while in fact this forms part of the 'validation stage'. The ICH guidelines achieved a great deal in harmonizing the definitions of required validation parameters, their calculation and interpretation. The International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has harmonized the requirements in two guidelines <sup>25–26</sup>. The first one summarizes defines validation and the

characteristics needed for various types of test procedures, the second one extends the previous test to include the experimental data required and some statistical interpretation.

#### **Accuracy and Precision**

Accuracy determines the closeness of agreement between a test result and the accepted reference value whereas precision gives the closeness of agreement between independent test results obtained under stipulated conditions.

#### **Specificity**

It is the ability of the method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix (that may be expected to be present in the sample matrix) under the stated conditions of the test (specificity = 100 % selectivity).

#### Linearity

The ability of the method to obtain test results proportional to the concentration of analyte (within a given range).

#### **Linear Range**

The range of concentrations or amounts of analyte over which the method gives test results proportional to the concentration of analyte or a linear calibration model can be applied with a known confidence level.

#### Ruggedness

The (intra - laboratory tested) behavior of an analytical process when small changes in the environmental and/or operating conditions are made. **Robustness** 

It is a measure of the capacity of the analytical procedure to remain unaffected by small but deliberate variations in method - performance parameters, which provides an indication of its reliability during normal usage.

#### **RESULTS AND DISCUSSION Reaction Mechanism**

It has been reported in the literature <sup>27</sup> that iodine is formed as a result of the interaction of a mixture of iodate and iodide with inorganic or organic acid in accordance with the equation.

$$5 \text{ I}^+ \text{ IO}_3^- + 6 \text{ H}^+ \rightarrow 3 \text{ H}_2\text{O} + 3 \text{ I}_2$$

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In aqueous medium, the iodide ions react with the liberated iodine to yield triiodide ion (I<sub>3</sub>)

 $I_2 + I^- \rightarrow I_3^-$ 

This detected in UV detector at 352 nm (Figure No.2). We realize that this reaction would be helpful for developing a simple spectrophotometric method for determination of vitamin C. Keeping this in mind, a mixture of potassium iodide and potassium iodate was allowed to react with vitamin C which yielded iodine. Then the liberated iodine reacted with the excess of iodide ion resulting in the formation of triiodide ion. The resulting triiodide ion oxidizes vitamin C in dehydroascorbic acid (Scheme I).

#### **Optimization of reaction conditions**

The different parameters affecting the development process were extensively studied to determine the optimum conditions for the assay procedures. The optimum values of the variables were maintained throughout the determination process.

#### Effect of the concentration of potassium iodate

The effect of the volume of potassium iodate (1000  $\mu$ g/ml) on the absorbance of the product was studied in the range of 0.1 - 1.2 ml. The absorbance increases with the increase in the volume of potassium iodate and became constant at 1.0 ml. Further addition of potassium iodate does not change in the absorbance and therefore, 100 µg/ml potassium iodate was chosen as an optimum value (Figure No.3).

#### Effect of the concentration of potassium iodide

The effect of the volume (2500 µg/ml) potassium iodide on the absorbance of the product was studied in the range of 0.1 - 2.4 ml, keeping the constant concentrations of vitamin C  $(3.5 \ \mu g/ml)$  and 100.0 µg/ml potassium iodate. The maximum absorbance was obtained with 2.0 ml; further addition caused no change on the absorbance. Thus, 500.0 µg/ml potassium iodide (Figure No.4) was used throughout the experiment.

#### Validation of proposed method

The linearity of the proposed method was constructed for vitamin C reference standard solution by plotting concentration of the compound versus the absorbance. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The accuracy and precision of the method was evaluated within the linear range based on the analysis of vitamin C reference standard samples and pharmaceutical formulations at 1.0, 2.0 and 3.0 µg/ml. Five independent analyses were performed at each concentrations level within one day (intraday precision) as well as for five consecutive days (interday precision). The accuracy was ascertained by recovery studies using the standard addition method. The proposed method was used for estimation of vitamin C from tablets after spiking with 50, 150 and 250 % additional pure drug. The amount of vitamin C was determined from the regression equation.

The absorbance – concentration plot for the proposed method was found to be rectilinear over the range of  $1.0 - 3.5 \,\mu\text{g/ml}$ . Linear regression analysis of calibration data gave the regression equation with correlation coefficients close to unity. Statistical analysis of regression lines were made regarding the standard deviation of residuals  $(S_{x/y})$ , standard deviation of slopes (S<sub>b</sub>) and standard deviation of intercepts (Sa) and the values are summarized in (Table No.1).

The within day precision assays were carried out through replicate analysis (n=5) of vitamin C corresponding to 1.0, 2.0 and 3.0 µg/ml. The interday precision was evaluated through replicate analysis of the pure vitamin C samples for five consecutive days at the same concentration levels as used in within day precision. The results of these assays are reported in Table No.2. As can be seen from Table No.2 that the recovery and relative standard deviation(RSD) values for within day precision were always lower than 100.10% and 0.80%; recovery and RSD values for interday precision were lower than 100.07% and 1.20%. The precision results are satisfactory.

The intraday and interday precision assays were also carried out for vitamin C in pharmaceutical formulations. The results are summarized in Table

No.3. As can be seen from Table 3 that the recovery and RSD values were in the ranges 99.85% to 100.07%; 0.133% to 1.20% and 99.90 to 100.10%; 0.167% to 1.001% for intraday and interday precision, respectively.

The proposed method was also used for estimation of vitamin C after spiking with 50%, 150% and 250% of additional pure vitamin C and the results are summarized in Table No.4. It shows that the % recovery and % RSD values were ranges 99.867 to 100.07 and 0.200 to 0.800, respectively.

The selectivity of the proposed method was ascertained by analyzing standard vitamin C in the present tablets such as lactose monohydrate, corn, starch, talc, vitamin  $B_1$ ,  $B_2$ ,  $B_6$ ,  $B_{12}$ ,  $D_3$ , aspartame, and biotin. It was observed that the excipients did not interfere with the proposed method.

S. No	Parameter	Range
1	Linear dynamic range (µg/ml)	1.0 - 3.5
2	Regression equation <sup>a</sup>	$Y = 1.001 \times 10^{-1} X + 1.4 \times 10^{-3}$
3	Slope	$1.001  imes 10^{-1}$
4	Intercept	$1.4  imes 10^{-3}$
5	Corrélation coefficient (r)	0.9999
6	S <sub>a</sub>	$9.13 imes10^{-4}$
7	S <sub>b</sub>	$2.60 imes10^{-4}$
8	LOD (µg/ml)	$2.46 imes10^{-1}$
9	LOQ (µg/ml)	$8.15 imes10^{-1}$
10	Variance $(S_0)^2$	$1.53  imes 10^{-6}$

#### Table No.1: Summary of optical of and regression characteristics the proposed method

<sup>a</sup>with respect to Y = a + bX, where X is the concentration in  $\mu g/ml$ , Y is the absorbance.

	Amount (µg/ml)		Recovery	RSD		C.L. <sup>c</sup>
Proposed methods			(%)	(%)	SAE <sup>b</sup>	
	Taken	Found ± SD <sup>a</sup>				
Intraday assay	1.000	$1.008\pm0.008$	100.10	0.80	0.004	0.010
	2.000	$1.999 \pm 0.006$	99.95	0.30	0.003	0.008
	3.000	$3.001 \pm 0.004$	100.03	0.13	0.002	0.005
Inter day assay	1.000	$0.999 \pm 0.012$	99.99	1.20	0.005	0.020
	2.000	$2.001 \pm 0.009$	100.05	0.45	0.004	0.010
	3.000	$3.002 \pm 0.006$	100.07	0.20	0.003	0.008

### Table No.2: Summary of accuracy and precision results in pure form

<sup>a</sup>Mean for five independent analyses. <sup>b</sup> SAE standard analytical error. <sup>c</sup>C.L. confidence limit at 95 % confidence level and four degrees of freedom (t = 2.776).

## Table No.3: Summary of accuracy and precision results in pharmaceutical formulations

	Amount (µg/ml)		RSD	Recovery		C.L. <sup>c</sup>
Proposed methods					SAE <sup>b</sup>	
Intraday assay	Taken	n Found ± SD <sup>a</sup>	(%)	(%)		
Ca – C 1000	1.00	$1.000\pm0.010$	1.000	100.00	0.005	0.012
	2.00	$1.999 \pm 0.006$	0.300	99.95	0.003	0.008
	3.00	$3.001 \pm 0.004$	0.133	100.03	0.002	0.005
Redoxon	1.00	$0.999 \pm 0.012$	1.200	99.90	0.005	0.002
	2.00	$2.001\pm0.009$	0.450	99.95	0.004	0.001
	3.00	$3.002\pm0.006$	0.200	100.07	0.003	0.008
Cal – C – Vita	1.00	$1.000 \pm 0.012$	1.200	100.00	0.005	0.002

	2.00	$1.997\pm0.008$	0.401	99.85	0.004	0.010
	3.00	$3.000\pm0.004$	0.133	100.00	0.002	0.005
Interday assay						
Ca – C 1000	1.00	$1.001 \pm 0.023$	0.200	100.10	0.001	0.003
	2.00	$1.998 \pm 0.007$	0.350	99.90	0.003	0.009
	3.00	$3.002 \pm 0.005$	0.167	100.07	0.002	0.006
Redoxon	1.00	$0.999 \pm 0.009$	0.901	99.90	0.004	0.011
	2.00	$1.998 \pm 0.010$	0.501	99.90	0.005	0.012
	3.00	$2.999 \pm 0.013$	0.433	99.97	0.006	0.016
Cal – C – Vita	1.00	$1.000 \pm 0.004$	0.400	100.00	0.002	0.005
	2.00	$2.000 \pm 0.011$	0.550	100.00	0.005	0.014
	3.00	$2.999 \pm 0.014$	0.467	99.97	0.006	0.017

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<sup>a</sup>SD. standard deviation. <sup>b</sup>SAE. standard analytical error. <sup>c</sup>C.L. confidence limit at 95 % confidence level and four degrees of freedom (t = 2.776)

Proposed method	Amount (µg/ml)			Recovery	RSD <sup>b</sup>	SAE <sup>c</sup>
	Taken	Added	Found ± SD <sup>a</sup>	(%)	(70)	
Ca – C 1000	1.00	0.50	$1.499 \pm 0.009$	99.93	0.600	0.004
	1.00	1.50	$2.501 \pm 0.010$	100.04	0.400	0.005
	1.00	2.50	$3.499 \pm 0.011$	99.97	0.314	0.005
Redoxon	1.00	0.50	$1.501 \pm 0.006$	100.07	0.400	0.003
	1.00	1.50	$2.498 \pm 0.008$	99.92	0.320	0.004
	1.00	2.50	$3.500 \pm 0.007$	100.00	0.200	0.003
Cal – C – Vita	1.00	0.50	$1.500 \pm 0.004$	100.00	0.267	0.002
	1.00	1.50	$2.499 \pm 0.013$	99.96	0.520	0.006
	1.00	2.50	$3.501 \pm 0.008$	100.03	0.229	0.004

#### Table No.4: Standard addition method

<sup>a</sup>Mean for 5 independent analyses. <sup>b</sup>RSD.relative standard deviation. <sup>c</sup>SAE standard analytical error.



Figure No.1: Structure of vitamin C

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Figure No.2: Absorption spectra of vitamin C  $(3.5 \ \mu g/ml) + \text{KIO}_3(100.0 \ (\mu g/ml))$  and KI  $(500.0 \ \mu g/ml)$  vs. blank KIO3  $(100.0 \ \mu g/ml)$  and KI  $(500.0 \ \mu g/ml)$  in distilled water



Figure No.3: Effect of the volume of KIO<sub>3</sub> (1000µg/ml); keeping constant concentration of vitamin C(3.5µg/ml) and KI (500µg/ml)

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FigureNo.4: Effect of the volume of KI (2500µg/ml); keeping constant 3.5µg/ml vitamin C and 100 µg/mlKIO<sub>3</sub>.

#### Scheme 1





#### CONCLUSIONS

The proposed method provides a simple, cost effective, fast and efficient method and does not require any laborious clean up procedure before measurement. In addition, the method has good linear dynamic range with good accuracy and precision. The method shows no interference from the common excipients and additives. This may help in analyzing affectivity of this vitamin in human beings during treatment. Therefore, it is concluded that the proposed method provides a rapid determination of vitamin C and can be frequently used in research and pharmaceutical laboratories. The proposed method can also be used as alternative method to reported ones for routine determination of vitamin C in bulk and pharmaceutical formulations.

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