A Colorimetric Method for Determination of Vitamin A and Carotene by Perchloric Acid.* (20571)

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In the course of studies of the mode of action of vit. A(1), it was noted that the unstable color which vit. A develops in the presence of many inorganic acids(2) often could be made more stable in a solution of amyl acetate. Perchloric acid was found to give the most intense color with vit. A and with carotene. On this basis a colorimetric method was devised, which makes possible the quantitative estimation of vit. A and of carotene in each other's presence.

Reagents. 1. Vit. A standard solution. Crystalline vit. A alcohol (Eastman-Kodak) or synthetic vit. A palmitate in corn oil (Hoffmann-LaRoche)† freshly dissolved in chloroform to a final concentration of 300 μg/cc and serially diluted. A standard curve is prepared with 0.1 ml volumes of these solutions, corresponding to 3-30 μg of vit. A. 2. Carotene standard solution. Carotene freshly dissolved in chloroform to final concentrations of 30-300 μg/ml. The standard curve is prepared with 0.1 ml amounts of these solutions. 3. Amyl acetate. 4. Concentrated hydrochloric acid. 5. Perchloric acid reagent. Three volumes of a concentrated solution of perchloric acid (70-72% perchloric acid) diluted with one part of distilled water. After cooling, the reagent is stored at ice box temperature. 6. Absolute alcohol.

Procedure. 1. Preparation of standard curves. Samples of 0.1 ml of the standard vit. A (3-30 μg) or carotene (3-30 μg) solutions are measured into test tubes and diluted with 1.6 ml amyl acetate. To this mixture 0.4 ml concentrated hydrochloric acid is added. The tubes are shaken and allowed to stand at room temperature for 4 to 5 minutes. This is followed by addition of 2 ml perchloric acid reagent. Carotene gives a bluish-green color with a broad maximum absorption between 725 and 760 mμ. With vit. A an immediate blue color develops which almost instantaneously changes to a purplish red tint with a maximum absorption between 520 and 530 mμ. The contents of the tubes are mixed with 0.3 or 0.4 ml absolute alcohol with vigorous shaking until the layers become miscible. The purplish-red color of vit. A, although losing in intensity, is visible for 25-30 minutes, after which it gradually turns yellow; the bluish-green color of carotene is somewhat more stable. The color was measured at the same time, 5 to 15 minutes after addition of the perchloric acid reagent. A mixture, containing 0.1 ml of chloroform in place of the vit. A or carotene solutions, prepared simultaneously with the other standard mixtures, serves as a blank. The blank mixture gradually turns yellowish on standing. Three standard curves are drawn: Vit. A is measured at 525 mμ, carotene at 525 and 750 mμ (Fig. 1). The curves follow the Beer-law in the above mentioned concentrations.

2. Estimation of unknown amounts of vit. A and carotene. A sample is diluted with chloroform to the proper range in which determinations are to be carried out. The subsequent steps in the color development are the same as those for the standard solutions.

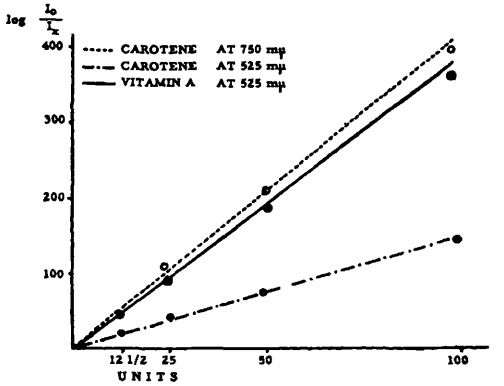

FIG. 1. Absorption curves of vit. A and carotene with perchloric acid.

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When only carotene was present, the readings were made at 750 mμ. When the unknown contained vit. A only, readings were made at 525 mμ and the amounts read from the standard curve for vit. A. In this case, determinations may be also carried out with the Klett-Summerson photoelectric colorimeter with filter No. 54. When both carotene and vit. A were present in the unknown, the following procedure was adopted: Readings were made at 525 mμ and 750 mμ. At 750 mμ the absorption of vit. A is negligible and readings made at this wave length permit the direct quantitative estimation of the carotene present in the unknown sample. The reading which corresponds to these amounts of carotene at 525 mμ is determined from the standard carotene curve (525 mμ) and subtracted from the reading obtained for the unknown at 525 mμ. The resulting figure then is read from the vit. A standard curve and gives the amounts of vit. A in the unknown sample. Comparison with Sobel's method(3) gave excellent agreement for vit. A in samples of fish liver oil. When 25 U of vit. A was added to a sample of fish liver oil containing 33 to 34 units, the recovered amounts were 58 to 60 units. Vit. D gave no color with the perchloric acid reagent.

Discussion. The color developed by vit. A in the presence of inorganic acids or some salts of strong acids (such as $SbCl_3$) is believed to be due to the formation of a colored vit. A cation(2,4). In the course of this cation formation, the absorption of vit. A shifts from the ultraviolet region to longer wave lengths. The first color shift to the blue, as occurs in the Carr-Price method, in the first stage of Sobel's method or in the present method, appears to be always very unstable. Only when a further shift to the purple region occurs, as in Sobel's or the present method, is there a fair degree of stability of color.

Among the advantages of the present method is the ready availability and stability of the inexpensive reagents which may be of value in large scale determinations. The color developed with perchloric acid follows the Beer-law over wider ranges than the colors obtained with previous methods. One of the main drawbacks of the method is its relatively low sensitivity, as compared with previous methods. The slow, but continuous fading of the developed color may lead to a lesser degree of accuracy than that obtainable with other methods, unless readings are made at exactly the same time after addition of the perchloric acid and under the same conditions.

Summary. A method is described for the quantitative colorimetric estimation of vit. A and carotene in each other's presence. The method is based on the fact that the purple-red color developed by vit. A (maximum absorption 525-530 mμ) and the bluish-green color developed by carotene (maximum absorption 725-760 mμ) in the presence of perchloric acid, may be stabilized in a solution of amyl acetate. In a mixture of vit. A and carotene, carotene may be estimated directly by determining its absorption at 750 mμ; the amounts of vit. A may be calculated by subtracting from the absorption, measured at 525 mμ, the absorption value which corresponds to carotene at this wave length.

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