Colorimetric Determination of Biotin

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Abstract □ A colorimetric method has been developed for the analysis of biotin in premixes with dicalcium phosphate. The method involves the oxidation of the sulfur atom in biotin to the sulfone with potassium iodate in an acid medium and the concurrent reduction of iodate to iodine. The liberated iodine is then extracted into cyclohexane and the absorbance measured at 520 mμ. The relationship between absorbance and the quantity of biotin reacted was found to obey Beer's law over the concentration range studied, 3–18 mg./g., and the extracted color is stable for several hours. The precision (*ts*:95% confidence limit) is equal to ±5.7%.

Keyphrases □ Biotin determination □ Dicalcium PO_4 preparation—biotin determination □ Colorimetric analysis—spectrophotometer

Although numerous microbiological methods (1–9) have been reported for the determination of biotin, the chemical analysis of biotin in dosage forms has been a difficult task. The current, widely accepted, procedure for the determination of biotin employs the microbiological assay. This procedure requires considerably more time for the determination of biotin than does the proposed colorimetric method; however it does have greater sensitivity. The colorimetric method may not be applicable to multivitamin products containing biotin at the microgram level or in the presence of reducing agents under the conditions of the procedure.

A literature search revealed a report on a colorimetric chemical assay of biotin (10). This report, although workable and reproducible, depended upon a reversal of binding in a dye-protein complex which is somewhat involved. A logically simpler and shorter approach seemed to be one which might take advantage of the lability of the sulfur atom toward oxidation. Since organic sulfides can be oxidized fairly readily to the sulfoxide (R_2SO) and further to the sulfone (R_2SO_2) it was anticipated that this technique could be employed as a means for a quantitative assay of biotin. Leitch (11) in an early report, described the quantitative oxidation of organic sulfides by sodium hypochlorite. This procedure employed a volumetric titration.

In the present authors' investigation it was found that biotin could be quantitatively determined by employing a colorimetric technique. The fused thiophane ring system present in the structure of biotin contains a sulfur atom which is susceptible to oxidation by potassium iodate in an acid medium. The iodine formed by the reduction of iodate is quantitatively extracted into cyclohexane and measured spectrophotometrically at 520 mμ.

EXPERIMENTAL

Reagents—*Potassium Iodate Reagent*—A 1% w/v solution of potassium iodate (analytical grade) is prepared in water.

Sulfuric Acid Reagent—A 30% v/v solution of sulfuric acid (analytical reagent grade) is prepared in water.

Cyclohexane[1]—Spectrophotometric grade.

Standard Biotin[2]—Reference standard biotin. Biotin may be recrystallized from a dimethylformamide–ethyl ether (1:1) solution. The crystals so obtained are dissolved in boiling water containing adsorption carbon.[3] The hot solution is then filtered through Whatman No. 2 paper and the colorless filtrate cooled in an ice bath. The pure biotin crystals are collected by means of vacuum filtration, washed with cold water, and dried at 105° for 4 hr.

Analytical Procedure[4]—Weigh accurately a sample of the preparation, equivalent to approximately 10 mg. of biotin, into a 50-ml. glass-stoppered centrifuge tube. Add 3.0 ml. of 1% potassium iodate reagent, mix well, then add 2.0 ml. of 30% sulfuric acid reagent. Pipet 20.0 ml. of cyclohexane into the tube and place the tube and contents into a 60° water bath, adjusting the water level in the bath to coincide with the aqueous phase in the centrifuge tube. Stopper each tube loosely in order to permit pressure release. Allow the centrifuge tube to remain in the bath for 30–40 min., then remove and immerse in an ice bath for several minutes. Shake the tube vigorously for 5 min., allow the contents to settle, and centrifuge at moderate speed for 5 min. Quantitatively transfer the cyclohexane layer into a 100-ml. volumetric flask. Repeat with 2 × 20-ml. portions of cyclohexane and combine all cyclohexane extracts in the 100-ml. volumetric flask. Attention should be given to the aqueous phase for evidence of any color of iodine. If iodine is still remaining in the bottom layer with the sample mass, a stirring rod should be used to agitate the solid phase until the iodine floats to the interface. A total of 3 × 20 ml. extracts are generally sufficient, however 4 × 20-ml. may be necessary if trouble is encountered with iodine being trapped in the solid phase. Adjust the volume of the cyclohexane extract to 100 ml. and determine the absorbance, A_p, of the solution at 520 mμ in a 5-cm. cell against a reagent blank.

Concomitantly carry out the reaction with reference standard biotin, weighing accurately approximately 10 mg. of the standard biotin into a glass-stoppered centrifuge tube and follow the procedure described above, beginning with "...add 3.0 ml. of 1% potassium iodate reagent." Designating the biotin standard in milligrams as C_s and the absorbance of the iodine solution produced as A_s, the biotin present in the preparation can be calculated from the equation:

$$\text{Biotin (mg./g.)} = \frac{A_p \times C_s}{A_s \times \text{sample weight (g.)}}$$

Alternately the biotin content of the sample may be calculated utilizing the absorptivity of iodine in cyclohexane at 520 mμ, since it has been demonstrated that a reproducible, stoichiometric release of iodine occurs as a function of biotin concentration.

RESULTS AND DISCUSSION

The concentration of biotin was found to be proportional to the absorbance measured at 520 mμ for the iodine formed by the reaction of biotin with potassium iodate in an acid medium.

Samples were assayed and the absorbances determined after varying the time intervals at the reaction temperature of 60°. The results, as shown in Fig. 1, indicated that at least a 30-min. time interval is required to obtain complete reaction. As can be seen, the reaction proceeds slowly even at 60°. This may be due in part to the low solubility of biotin in the reaction medium. Care should be taken that the temperature of the water bath be controlled at

[1] Obtainable from Matheson, Coleman & Bell.
[2] Hoffmann-La Roche, Inc., Nutley, N. J.
[3] Norit, American Norit Co., Inc., Jacksonville, Fla. 32208
[4] A Beckman DU spectrophotometer was employed for all absorbance measurements which were carried out in 5-cm. silica cells.

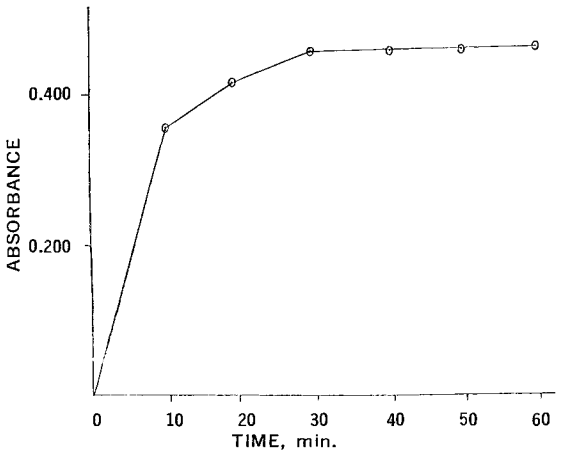

Figure 1—*Reaction time at 60°.*

60° since higher temperatures may result in a loss of iodine and consequently low results.

Considering biotin as a compound in the general class of $\begin{matrix} R \\ R_1 \end{matrix}\!\!>\!S$, the two possible oxidation reactions that could occur would produce either the sulfoxide or the sulfone if exact stoichiometry is assumed as shown in Eq. 1 or Eq. 2. The biotin moiety may be shown as R_2S for simplicity

$$5R_2S + 2IO_3^- + 2H^+ \rightarrow 5R_2SO + I_2 + H_2O \quad \text{(sulfoxide)} \qquad \text{(Eq. 1)}$$

or

$$5R_2S + 4IO_3^- + 4H^+ \rightarrow 5R_2SO_2 + 2I_2 + 2H_2O \quad \text{(sulfone)} \qquad \text{(Eq. 2)}$$

Stoichiometrically either Eq. 1 or Eq. 2 could be occurring. Experimentally it was found that 1 mmole of biotin yielded 0.35 mmole of iodine indicating that Eq. 2, yielding the sulfone was the major oxidation reaction. The results are reproducible and quantitative. Recovery data of known samples, analyzed by the proposed procedure, was 98 ± 1%.

Six replicates of a dicalcium phosphate preparation, containing 11 mg. of biotin per gram, gave a mean of 10.82 mg./g. with a relative *SD* from the mean of 0.24 mg. The precision (*ts*:95% confidence limit) was ±0.62 (Table I). Data obtained, comparing the colorimetric method with the microbiological method, on fourteen different samples of premix is presented in Table II.

Table I[a]—Biotin Replicate Data from a Representative Premix

Trials	Assay, mg./g.
1	10.48
2	10.92
3	10.95
4	10.55
5	11.04
6	10.97
Mean 10.82	
SD ±0.24	
Precision (*ts*:95% confidence limit), ±0.62	

[a] Theoretical concentration: 11.0 mg./g.

Table II—Comparative Recovery of *d*-Biotin from a 1% Premix

Sample No.	Colorimetric Method, %	Microbiological Method, % (12)
1	1.02	1.04
2	1.02	1.10
3	0.92	1.17
4	1.08	1.16
5	1.04	1.19
6	1.04	1.13
7	1.12	1.13
8	1.12	1.13
9	0.98	1.12
10	1.02	1.09
11	0.92	1.00
12	1.08	1.04
13	1.05	1.03
14	1.07	1.12

It should be noted that the method is relatively nonspecific and probably would not be stability-indicating. In addition, any compound susceptible to oxidation by potassium iodate in an acid medium (such as other sulfur-containing compounds as well as 1,2-diols) would interfere with the determination of biotin using this procedure and suitable separation steps such as ion exchange chromatography would be required.

SUMMARY

A colorimetric method has been developed for the determination of biotin in premixes with dicalcium phosphate. This procedure was found to be suitable for the analysis of biotin at a concentration of 10 mg./g. The reaction consists in the oxidation of biotin, to the sulfone, with potassium iodate in an acid medium and the concurrent reduction of iodate to iodine. The precision of the method (*ts*:95% confidence limit) is ±5.7%.

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