Rapid Determination of the Relative Purity of Vitamin B_{12} (Cyanocobalamin) in Pharmaceutical Products

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The validity of the spectrophotometric procedure for determining the purity of cyanocobalamin in pharmaceutical products offered for injection is further demonstrated. This procedure is based on the ratio of absorptivities at 341 and 376 mμ. Samples of cyanocobalamin injection which met U. S. P. XVI specifications for purity and which were authenticated by inspectional evidence obtained by the Food and Drug Administration served as the reference standards.

In a previous report (1) a rapid spectrophotometric method was proposed to determine the relative purity of cyanocobalamin in pharmaceutical products involving the use of the ratio of absorptivities at 341 and 376 mμ. The validity of the method when applied to solutions containing only cyanocobalamin was established by using reference samples of cyanocobalamin U. S. P. (2).

With commercial preparations such as cyanocobalamin injection U. S. P. where authentic injections were unavailable, the validity of the method was established by use of the purity index. This index, by definition, is the ratio on a percentage basis obtained by dividing the cyanocobalamin content determined by the radioisotope tracer method (3) by the cyanocobalamin content determined by the U. S. P. spectrophotometric method (2). Arbitrarily, an injection giving an index of 95% or more denoted that the cyanocobalamin used was of U. S. P. quality and that the injection was likewise of U. S. P. quality. It is the purpose of this report to establish the validity of the spectrophotometric method for commercial cyanocobalamin injections with greater certainty than that previously demonstrated in which the somewhat arbitrary purity index reference was used. The reference solutions used in this report consisted of cyanocobalamin injections which were of U. S. P. quality since they met all the requirements of the U. S. P. XVI, including in particular the test for cyanocobalamin solids, and were authentic as demonstrated by inspectional evidence.

To ascertain that samples of cyanocobalamin injection U. S. P. are made from cyanocobalamin U. S. P., the U. S. P. XVI (2) now contains under cyanocobalamin injection a test for "Limit of Cyanocobalamin Solids." Essentially this test determines the purity of the cyanocobalamin used in the injection. Specifications allow an upper limit of 1.2 mg. cyanocobalamin solids per mg. of cyanocobalamin. Solids in excess of this limit would indicate that impure cyanocobalamin had been used as an ingredient.

It might be pointed out, however, that this official solids test requires long periods of drying of the total solids and a gravimetric chloride determination which delays results for possibly two to three working days. On the other hand, the spectrophotometric method normally requires less than one hour and, because of its rapidity, we have used it as a sorting procedure in the Nutrition Division of the Food and Drug Administration. As previously reported, the proposed spectrophotometric method for cyanocobalamin injections established the ratio of absorptivities at 341 and 376 mμ of not more than 1.02 for injections of U. S. P. quality. Ratios above 1.02 would indicate that the product in question contained cyanocobalamin of less than U. S. P. XVI purity.

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EXPERIMENTAL

Eleven commercially prepared products of cyanocobalamin injection U. S. P. containing 1,000 mcg. cyanocobalamin per ml. and representing 11 manufacturers were selected as samples. The authenticity of each product was established through inspection by the Food and Drug Administration. After each sample was collected from an interstate shipment, a factory inspection was made to ascertain in particular that cyanocobalamin U. S. P. had been used. These samples were examined for cyanocobalamin solids by the method in U. S. P. XVI and the results are given in Table I. Since none of the observed values exceed 1.20 mg. cyanocobalamin solids per mg. of cyanocobalamin it can be concluded that, as measured by this test, all samples were of U. S. P. quality and were made from cyanocobalamin U. S. P.

Table I also shows the ratio of absorptivities at 341 and 376 mμ for the authentic samples determined as described in the previous report (1). Since all ratios are less than the prescribed maximum of 1.02, the conclusion can be drawn that all injections were made from cyanocobalamin of U. S. P. quality; and that the injections are also of U. S. P. quality. These data are thus in conformity with the data from the solids test demonstrating the validity of the ratio test.

TABLE I.—CYANOCOBALAMIN SOLIDS AND ABSORPTIVITY RATIOS OF CYANOCOBALAMIN INJECTIONS U. S. P.

Sample	Purity Index	Cyanocobalamin Solids, mg./mg. Cyanocobalamin		Absorptivity Ratio 341 mμ/376 mμ
1	100.5	1.10,	1.06	0.985
2	97.4	1.11	..	0.989
3	98.7	1.13	..	0.986
4	103.7	1.09	..	0.985
5	...	1.10	..	1.014
6	...	1.10	..	1.000
7	...	1.13	..	0.990
8	...	1.16[a]		1.013
9	...	1.08	..	0.989
10	...	1.11,	1.12	0.984
11	...	1.08,	1.14	0.985

[a] Declared esters of *para*-hydroxybenzoic acid in this injection were removed as directed by the U. S. P. XVI (2) before proceeding with solids determination.

TABLE II.—CYANOCOBALAMIN SOLIDS AND ABSORPTIVITY RATIOS OF CYANOCOBALAMIN INJECTIONS MADE FROM IMPURE CYANOCOBALAMIN

Sample	Purity Index	Cyanocobalamin Solids, mg./mg. Cyanocobalamin		Absorptivity Ratio 341 mμ/376 mμ
1	96.9	1.42,	1.46	1.063
2	91.3	1.81,	1.83	1.085
3	91.8	3.07,	3.08	1.139
4	..	2.78,	2.81	1.170
5	..	1.39,	1.33	1.036
6	..	1.39,	1.41	1.047
7	..	1.37,	1.38	1.061
8	..	1.41	..	1.051
9	..	1.36,	1.36	1.040

The purity index values shown in Table I (and also those in Table II) were obtained as described in the previous report (1). All four values shown in Table I are in excess of 95% indicating, by definition, that all the injections involved were of U. S. P. quality, a conclusion which is in conformity with that obtained from the two other tests shown in Table I.

It is interesting to note that if the authenticity of the samples as determined by inspectional evidence is accepted as valid, it appears that the data recorded here for cyanocobalamin solids for all the samples of Table I support the 1.20 limit as a reasonable selection.

Table II lists nine samples of commercially prepared injections of cyanocobalamin which were sampled while in interstate commerce and represent the output of four manufacturers. Although the products were represented to be of U. S. P. quality, inspectional evidence obtained by the Food and Drug Administration indicated that all injections were made from impure cyanocobalamin sources such as cobalamin concentrate N. F. (4) or similar products. The data shown for cyanocobalamin solids all exceed 1.20 mg./mg. cyanocobalamin, indicating that the injections were not of U. S. P. quality and could not have been made from cyanocobalamin U. S. P. The ratios of absorptivities at 341 and 376 mμ also exceeded 1.02 in all samples, permitting one to draw the same conclusion as that obtained from the solids test. Thus, with injections that do not conform to U. S. P. XVI requirements the ratio data parallel the cyanocobalamin solids data to a remarkable degree.

The purity index of sample No. 1 of Table II indicates by definition that this injection is of U. S. P. quality, a conclusion different from that obtained from the results of the other two tests of Table II. It is believed that the singular composition of this injection was responsible for the variance in conclusions because the corresponding purity index values were 87.2 and 86.2% when cyanocobalamin was determined by a counter current distribution method (5) rather than by the tracer method, indicating the presence of impure cyanocobalamin in the injection.

CONCLUSION

A study has been made of 20 cyanocobalamin injection samples consisting of 11 authentic samples of U. S. P. quality and nine samples of lower quality.

For each of the 20 samples the ratio of absorptivity at 341 and 376 mμ indicated, with approximately the same degree of certainty as did the U. S. P. XVI cyanocobalamin solids test, whether or not the injection was of U. S. P. quality and whether or not cyanocobalamin U. S. P. had been used in preparing the injection. This agreement of the two methods further establishes the validity of the spectrophotometric ratio method.

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Book Notices

Biochemical Preparations. Vol. 8. Edited by ALTON MEISTER. John Wiley & Sons, Inc., 440 Park Avenue South, New York 16, N. Y., 1961. ix + 146 pp. 15 × 22.5 cm. Price $6.25.

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