TABLE VI—COMPARISON OF ASPIRIN TABLET STABILITY TESTING RESULTS AT 50°C. AND 81.2% RELATIVE HUMIDITY, BASED ON THE DETERMINATION OF ASPIRIN AND SALICYLIC ACID CONTENT OF THE TABLETS[a]

Time, Days	Aspirin Content Based on Analysis of Aspirin, %	Aspirin Content Based on Analysis of Salicylic Acid, %	Error Due to Sublimation of Salicylic Acid, %
0	100	100	0
15	99.1 ± 0.038	99.1 ± 0.028	0
30	98.4 ± 0.042	98.8 ± 0.215	0.4
45	97.2 ± 0.123	98.7 ± 0.075	1.5
60	97.1 ± 0.178	98.6 ± 0.023	1.5
98	95.0 ± 0.288	97.9 ± 0.311	2.9

[a] Each value is the average of 4 determinations recorded with ±1 standard deviation.

be interpreted as a trend toward equilibrium within the tablet (2). Judging from the lower curve, no such trend toward equilibrium is apparent. The data pertaining to Fig. 5 is summarized in Table VI.

SUMMARY AND CONCLUSIONS

Previously reported studies of the stability of aspirin-containing solids have utilized the salicylic acid content of the solids as a measure of aspirin decomposition. The underestimation in the extent of decomposition of the aspirin which can result from such a practice is clearly demonstrated by the results of the present work. The error can be expected to become increasingly serious with storage time and the severity of the temperature and humidity conditions under which the solids are stored. This error can be circumvented by employing a method of determining decomposed aspirin which is independent of the loss of salicylic acid from the solid due to sublimation. Since aspirin was found not to sublime, the determination of its residual content in a solid is a valid means of gauging the stability of the formulation. A method of analysis for aspirin with an accuracy of at least 1.5% was developed for application to testing of aspirin stability in solid dosage forms.

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Keyphrases

Aspirin stability—salicylic acid sublimation
Salicylic acid sublimation—aspirin tablets
Temperature effect—aspirin tablets stability
UV spectrophotometry—analysis

Stability of Cyanocobalamin in Film-Coated Multivitamin Tablets

By JAMES T. JACOB, ROBERT J. NESSEL, and JACK BLODINGER

Multivitamin tablets containing each of two commercially available protected forms of cyanocobalamin showed considerable loss of vitamin B_{12} after exposure to methanol vapor for 1 month at room temperature. Other solvents commonly used in film coating such as acetone, *n*-butanol, butyl acetate, isopropanol, and methylene chloride did not affect the stability of vitamin B_{12}. Tablets containing vitamins B_1, B_{12}, ascorbic acid, and niacinamide, alone and in combination with one another after exposure to methanol vapor at room temperature showed considerable loss of vitamin B_{12} only in presence of ascorbic acid and/or niacinamide.

THERE ARE SEVERAL reports in the literature on the stability of cyanocobalamin (vitamin B_{12}) in liquid multivitamin preparations. Decomposition products of other vitamins, pH, heat, and light can in most cases contribute to the degradation of vitamin B_{12}. Feller and Macek (1) reported that decomposition of vitamin B_{12} occurs at elevated temperatures in the presence

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of thiamine decomposition products. Doerge, *et al.* (2) found that a thiol-containing degradation product of thiamine hydrochloride may be responsible for the losses of vitamin B_{12} potency during storage. Bartilucci *et al.* (3) showed that in liquid preparations, dehydroascorbic acid, the first decomposition product of ascorbic acid, caused greater instability of vitamin B_{12} than ascorbic acid.

Cyanocobalamin has been found to be stabilized in solid dosage forms, such as multivitamin tablets by the incorporation of the vitamin in the sugar coating, thus separating it from the other active components. However, with the increasing use of film-coating, this method is not applicable. For this reason, the use of a protected form of the vitamin is a necessity. Two such forms commercially available are cyanocobalamin dispersed in gelatin[1] and cyanocobalamin adsorbed on resin.[2]

The process of film-coating involves spraying a solution of a film-forming polymer in single or mixed organic solvents onto the moving bed of tablets or within an air suspension column. The solvents are then removed by intermittent drying of the tablets. The total time for film-coating varies, depending upon the type of film-forming polymers and solvents used, thickness of the film, number of tablets coated, and the process used. It is quite possible for the solvent(s) to penetrate into the tablet core during the long exposure. Holl *et al.* (4) reported that tablets that were film-coated with a solution of cellulose acetate phthalate, even after considerable drying, retained residual amounts of acetone. Other solvents commonly used in film-coating are methanol, methylene chloride, chloroform, butanol, and isopropanol.

Vitamin B_{12} dispersed in gelatin has shown very good stability in uncoated multivitamin tablets. However, because of an unexpected loss in potency observed when incorporated in tablets that were film-coated, this investigation was undertaken. The purpose of this investigation was to determine whether the solvents used in film-coating may be the cause of the aforementioned instability. For this study, solvent effects were determined on two commercially available protected cyanocobalamin forms.

EXPERIMENTAL

Materials—Acetone,[3] ascorbic acid, USP, butyl acetate,[4] *n*-butyl alcohol,[3] chloroform,[3] 1% cyanocobalamin dispersed in gelatin, 1% cyanocobalamin adsorbed on resin, isopropyl alcohol,[3] anhydrous methanol,[3] methylene chloride,[3] niacinamide, USP, thiamine mononitrate, USP.

TABLE I—COMPOSITION OF MULTIVITAMIN TABLETS

Ingredient	Potency per Tablet
Vitamin A acetate	5000 units
Vitamin D_2	400 units
Thiamine mononitrate, USP	1.4 mg.
Riboflavin, USP	2.0 mg.
Ascorbic acid[a]	70.0 mg.
Niacinamide[a]	22.0 mg.
d-Calcium pantothenate, USP	10.0 mg.
Cyanocobalamin[b]	4.0 mcg.
Vitamin E succinate	15 units
Folic acid	0.1 mg.
Dicalcium phosphate	20.0 mg.
Microcrystalline cellulose[c]	30.0 mg.
Magnesium stearate	2.5 mg.
Stearic acid	5.0 mg.
Talc	10.0 mg.

[a] In the form of Merpress—registered trademark for niacinamide ascorbate, Merck & Co., Inc. Rahway, N. J. [b] Added as 1% cyanocobalamin dispersed in gelatin; second batch contained the same quantity in the form of 1% cyanocobalamin adsorbed on resin. [c] Marketed as Avicel by American Viscose Co., Marcus Hook, Pa.

Preparation of Tablets—Two 1,000-tablet batches of a full formula multivitamin were prepared by a direct compression method using 0.79-cm. ($^5/_{16}$-in.) dies and standard concave punches on a Manesty single-punch machine. One batch contained vitamin B_{12} in the form of cyanocobalamin dispersed in gelatin and the other, in the form of cyanocobalamin adsorbed on resin. The quantitative formula is shown in Table I.

Immersion in Solvents—Ten tablets were immersed for exactly 60 sec. in a beaker containing each of the following solvents: acetone, butyl acetate, *n*-butyl alcohol, chloroform, isopropyl alcohol, methyl alcohol and methylene chloride. The solvent was then poured off, the tablets were air dried for 30 sec. and one-half of the tablets were stored at room temperature and the rest at 50°. After 1 month, the tablets were removed and were assayed for vitamin B_{12} content.

Exposure to Solvent Vapors—Twenty multivitamin tablets were placed in loosely capped 20-ml. amber bottles, and stored in desiccators containing each of the solvents used in the immersion test at room temperature. After storage for 1 month, the tablets were assayed for vitamin B_{12} content.

Solubility Test—The dissolution of cyanocobalamin from the two protected forms in methanol was determined as follows: 200 mg. of each powder was weighed into 125-ml. amber colored conical flasks. Fifty milliliters of anhydrous methanol was added and the flasks shaken on a mechanical shaker[5] for 48 hr. at room temperature. The solution was filtered and the absorbance of the filtrate was measured at 361 mμ on a Cary II spectrophotometer using methanol as the reference solvent. The quantity of dissolved cyanocobalamin was calculated from a standard curve of cyanocobalamin solution in methanol.

Assay Methods—The tablets were assayed for vitamin contents using standard assay methods.

[1] Marketed as Stabicote by Merck & Co., Inc., Rahway, N. J.
[2] Marketed as Stablets by Chas. Pfizer & Co., Inc., New York, N. Y.
[3] Reagent Grade, Merck & Co., Inc., Rahway, N. J.
[4] Matheson, Coleman and Bell, Norwood, Ohio.
[5] Burrel Wrist Action shaker, Pittsburgh, Pa.

TABLE II—% LOSS OF VITAMIN B_{12} FROM MULTIVITAMIN TABLETS EXPOSED TO VARIOUS SOLVENT VAPORS FOR 1 MONTH AT ROOM TEMPERATURE

Batch No.	Acetone	*n*-Butanol	Butyl Acetate	Chloroform	Isopropanol	Methylene Chloride	Methanol
1[a]	No loss	No loss	1.2	No loss	No loss	No loss	24.5
2[b]	No loss	0.5	5.7	0.5	No loss	No loss	26.5

[a] Contained B_{12} as 1% cyanocobalamin dispersed in gelatin. [b] Contained B_{12} as 1% cyanocobalamin adsorbed on resin.

RESULTS AND DISCUSSION

Immersion in Solvents—The time for immersion of the tablets in the solvents was limited to 1 min. so that the tablets would not be soaked with the solvents. The assay results showed little or no loss of vitamin B_{12} potency after storage either at room temperature or at 50° for 1 month. The concentration of the solvents retained in the tablets may not have been quite sufficient to cause any stability problem for vitamin B_{12}.

Exposure to Solvent Vapors—The tablets stored in desiccators containing *n*-butyl alcohol, butyl acetate, chloroform, isopropanol, and methylene chloride showed no loss of vitamin B_{12} potency, whereas tablets similarly exposed to methanol vapors showed considerable loss (Table II). Because of this loss, the dissolution of cyanocobalamin in methanol from its two protected forms was determined in order to consider the possibility of extraction of vitamin B_{12} from the protective matrix into the tablet. Twenty-five percent of vitamin B_{12} dissolved in methanol from the gelatin-dispersed form, while 55% of the vitamin leached from the resin adsorbate. Exposure of the two protected forms of vitamin B_{12} *per se* to methanol vapor for 1 month showed no loss from the gelatin-dispersed form, whereas 20% loss was observed from the resin adsorbate. This loss of vitamin B_{12} may be attributed to the fact that methanol vapor easily penetrated the resin adsorbate, which remained as a powder, whereas the gelatin-dispersed powder solidified in the bottle thereby preventing any penetration of methanol vapor. Since in aqueous solutions, degradation products of ascorbic acid, especially dehydroascorbic acid, are known to adversely affect the stability of vitamin B_{12} (4), it was necessary to determine whether this or any other vitamin in presence of methanol vapor may have any deleterious effect on the stability of vitamin B_{12}. For this purpose, tablets were prepared with ascorbic acid, niacinamide, thiamine mononitrate, cyanocobalamin dispersed in gelatin, and cyanocobalamin adsorbed on resin, alone and in combination with one another. The potency of each vitamin and the excipient quantities were maintained at the same level as in the multivitamin tablets except that spray-dried lactose was used to adjust the weight to that of the multivitamin tablets. All tablets were formulated so as to be of same size, weight, and hardness. These tablets were then exposed to methanol vapor by storage in desiccators for 1 month at room temperature.

TABLE III—COMPOSITION[a] AND POTENCY LOSS OF INDIVIDUAL VITAMINS FROM TABLETS EXPOSED TO METHANOL VAPOR FOR 1 MONTH AT ROOM TEMPERATURE

Batch No.	Potency of Each Tablet: B_{12}, mcg.	B_1, mg.	Ascorbic Acid, mg.	Niacinamide, mg.	% Loss
3	4.0[b]	—	—	—	0.0
4	4.0[c]	—	—	—	5.0
5	—	1.4	—	—	18.0
6	—	—	70.0	—	4.4
7	—	—	—	22.0	0.0

[a] In addition to the vitamin, each tablet contained the same quantities of excipients as in the multivitamin tablet (Table I) and sufficient quantity of spray-dried lactose to adjust the weight to that of the multivitamin tablet. [b] As 1% cyanocobalamin dispersed in gelatin. [c] As 1% cyanocobalamin adsorbed on resin.

A study of Table III shows that methanol vapor did not cause an appreciable decrease in the potencies of protected vitamin B_{12} (both forms), ascorbic acid, and niacinamide when these vitamins were present alone in the tablets. The effect was more pronounced on vitamin B_1 tablets which lost 18% of the vitamin (see Batch No. 5). Table IV shows that there was only a small loss of ascorbic acid, but its presence in the tablet caused considerable loss of vitamin B_{12}. There was also a significant loss of vitamin B_1 from the tablets (see Batch Nos. 10 and 11). Table V shows that tablets containing vitamins B_1-B_{12}-niacinamide combinations had little or no loss of either vitamin B_1 or niacinamide in presence of methanol vapor. Only one batch (Batch No. 16) lost a substantial amount of vitamin B_{12}. The large loss of vitamin B_1 did not cause any stability problem for vitamin B_{12} when these two vitamins were present together in the tablet (see Batch Nos. 12 and 13). Since this study was limited only to the assay of vitamin contents after exposure to methanol vapors, no attempt was made to isolate the degradative products of the vitamins, especially those of ascorbic

TABLE IV—COMPOSITION[a] AND POTENCY LOSS OF VITAMINS FROM B_1-B_{12}-ASCORBIC ACID TABLETS EXPOSED TO METHANOL VAPOR FOR 1 MONTH AT ROOM TEMPERATURE

Batch No.	Potency of Each Tablet: B_1, mg.	B_{12}, mcg.	Ascorbic Acid, mg.	% Loss: B_1	B_{12}	Ascorbic Acid
8	—	4.0[b]	70.0	—	14.5	3.7
9	—	4.0[c]	70.0	—	19.4	8.1
10	1.4	4.0[b]	70.0	17.6	7.3	5.2
11	1.4	4.0[c]	70.0	13.0	17.0	3.0

[a] In addition to the vitamins, each tablet contained the same amount of excipients as in the multivitamin tablets (Table I) and sufficient quantity of spray-dried lactose to adjust the weight to that of the multivitamin tablet. [b] As 1% cyanocobalamin dispersed in gelatin. [c] As 1% cyanocobalamin adsorbed on resin.

TABLE V—COMPOSITION[a] AND POTENCY LOSS OF VITAMINS FROM B_1-B_{12}-NIACINAMIDE TABLETS EXPOSED TO METHANOL VAPOR FOR 1 MONTH AT ROOM TEMPERATURE

Batch No.	Potency of Each Tablet			% Loss		
	B_1, mg.	B_{12}, mcg.	Niacinamide, mg.	B_1	B_{12}	Niacinamide
12	1.4	4.0[b]	—	12.6	2.4	—
13	1.4	4.0[c]	—	14.2	4.9	—
14	1.4	—	22.0	0.0	—	0.0
15	1.4	4.0[b]	22.0	1.1	4.9	3.8
16	1.4	4.0[c]	22.0	1.1	14.6	4.0

[a] In addition to the vitamin, each tablet contained the same quantities of excipients as in the multivitamin tablet (Table I) and sufficient quantity of spray-dried lactose to adjust the weight to that of the multivitamin tablet. [b] As 1% cyanocobalamin dispersed in gelatin. [c] As 1% cyanocobalamin adsorbed on resin.

acid and vitamin B_1. No quantitative relationship between loss of vitamin B_{12} and loss of other vitamins could be observed. Nevertheless, it is quite apparent that methanol vapor when allowed to penetrate multivitamin tablets can cause loss of potency of individual vitamins, especially vitamins B_1 and B_{12}. It can be postulated that methanol vapor causes degradation of either ascorbic acid or vitamin B_1, or both, the degradation products of which in turn influence stability of vitamin B_{12}. This fact should be taken into consideration when methanol is used as a solvent in film coating of multivitamin tablets.

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Keyphrases

Cyanocobalamin (vitamin B_{12})—stability

Film-coated multivitamin tablets—vitamin B_{12} stability

Methanol vapor effect—vitamins B_1, B_{12} stability

Ascorbic acid effect—vitamins B_1, B_{12} stability

Fluorometric Determination of Norgestrel and Structurally Related Steroids

By L. F. CULLEN, J. G. RUTGERS, P. A. LUCCHESI, and G. J. PAPARIELLO

A sensitive procedure, based on sulfuric acid-induced fluorescence, has been developed for the analysis of norgestrel (*dl*-13-ethyl-17α-ethinyl-17-hydroxygon-4-en-3-one) in tablets of low dosage, *i.e.*, 15–75 mcg. Optimum conditions for fluorescence have been established and the fluorescent properties of structurally related steroids studied to determine the selectivity of the reaction and mechanism of fluorescence formation. The reaction is specific for Δ^4-3-ketosteroids which have both a 17β-hydroxyl and 17α-alkyl or alkyne substitution and $\Delta^{1,3,5(10)}$-triene-3-ol steroids. A two-step mechanism is tentatively explained on the basis of the effects of temperature, time, initial acid concentration, and subsequent dilution with water on fluorogen development. Specificity of the method with respect to the analysis of intact norgestrel in the presence of its photochemical and thermal degradation products was demonstrated by comparison to quantitative thin-layer chromatography values. This procedure has been automated to permit unit dose analysis. This automated procedure is capable of analyzing 15 samples per hour with a relative standard deviation of ±1.4 percent at the 50-mcg. level.

THE SYNTHESIS of a new, extremely potent progestational steroid, norgestrel (*dl*-13-ethyl-17α-ethinyl-17-hydroxygon-4-en-3-one), and subsequent formulation of this steroid in tablets of low dosage, *i.e.*, 15–75 mcg. per tablet, had presented a challenging analytical problem. A sensitive, accurate, and rapid procedure was desired for content uniformity testing of the dosage form.

Steroids structurally related to norgestrel, having a characteristic Δ^4-3-keto group in the

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