Stability of Vitamin C (Ascorbic Acid) in Tablets

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Abstract □ Stability of ascorbic acid (vitamin C) in various tablet formulations and the nature and extent of formation of decomposition products (dehydroascorbic acid, diketogulonic acid, and oxalic acid) were determined under normal conditions of storage and in simulated use tests. IR spectrophotometric, colorimetric, fluorometric, titrimetric, polarographic, and chromatographic methods were applied. Recent implications concerning the instability of ascorbic acid in tablets and the potentially harmful nature of the breakdown products are shown to be unfounded. Under normal storage conditions, commercial-type ascorbic acid tablets are stable for over 5 years (>95% potency retention). The amounts of all three breakdown products formed under the various storage conditions constitute a small percentage of the ascorbic acid content and pose no dietary hazard. IR spectroscopy was inadequate as a quantitative method for evaluating ascorbic acid potency in tablet formulations. The official titration methods and TLC, colorimetric, and polarographic determinations correlate well and define accurately the stability of ascorbic acid in these dosage forms.

Keyphrases □ Ascorbic acid—stability in various tablet formulations, nature and extent of formation of decomposition products □ Vitamins—ascorbic acid, stability in various tablet formulations, nature and extent of formation of decomposition products □ Stability—ascorbic acid in various tablet formulations, nature and extent of formation of decomposition products □ Decomposition products, ascorbic acid—various tablet formulations, nature and extent of formation

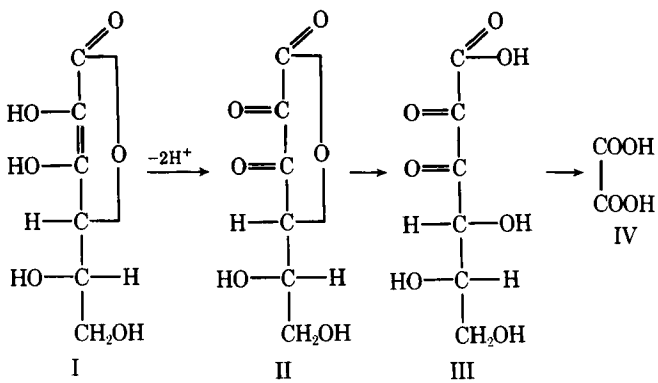
At the Second Conference on Vitamin C (1) in October 1974, international authorities in the fields of human biochemistry, physiology, pharmacology, and clinical medicine presented papers demonstrating that ascorbic acid (vitamin C) is essential not only to prevent scurvy but also to protect the body against drugs (2), environmental chemicals (3–7), and production of certain tumors (8, 9), to make energy available at the cellular level (10), to protect vascular systems, including protection against cholesterol (11, 12), to increase athletic performance (13), and to ameliorate the severity of the common cold (14–16). It was shown that the need for ascorbic acid is increased in women taking oral contraceptives (17), in smokers (18), and in persons with intestinal malabsorption (19). It was shown too that ascorbic acid supplements are safe (20). Tablets of ascorbic acid are the dosage form most commonly used.

Recently, Wilk (21) claimed, on the basis of very limited data, that extensive degradation of ascorbic acid occurred in tablets during "household storage." He alleged that existing analytical methodology is nonspecific and that IR spectroscopy is a better measure of ascorbic acid stability. It was also alleged that degradation products of ascorbic acid (I, Scheme I) in aged tablets, as well as normal tablet excipients, are potentially harmful to the consumer. These products include dehydroascorbic acid as an alleged diabetogenic agent, oxalate from aged tablets as a possible contributor to kidney stone formation, and the excipients sucrose, starch, and dextrin as weight-increasing substances and lactose as undesirable for individuals intolerant to that sugar. None of these was actually measured by Wilk (21).

The present paper provides more comprehensive data on the stability of ascorbic acid tablets under various storage conditions, as well as actual measurements of dehydroascorbic acid (II), diketogulonic acid (III), and oxalic acid (IV) (Scheme I) in various tablets. Diverse analytical methods are available for the determination of ascorbic acid. For tablets where ascorbic acid is the only reducing agent present, direct oxidimetric titration is classical. Official methods involve titration with iodine (22, 23) or 2,6-dichloroindophenol (23, 24). Chloramine-T (25) and *N*-bromosuccinimide (26, 27) also have been used as titrants.

Colorimetric or spectrophotometric methods for assay of ascorbic acid have been based on reactions with 2,6-dichloroindophenol (28–30), diazotized 2-nitroaniline (31, 32), diazotized 4-methoxy-2-nitroaniline (33, 34), 2,4-dinitrophenylhydrazine (35), 4-nitrobenzenediazonium fluoroborate (36), phosphomolybdate complex (37), 2,3,5-triphenyltetrazolium chloride (38), and phenylhydrazium chloride (39). A highly specific, microfluorometric assay for ascorbic acid, based on the reaction of dehydroascorbic acid with *o*-phenylenediamine plus a borate blank was described (40).

Other reported methods used for the assay of ascorbic acid include polarography (41, 42), GLC (43, 44), and thermometric measurement (45). Several chromatographic separation techniques, prior to titration or colorimetric assay of ascorbic acid, have been utilized to increase the specificity of the determination. These techniques include separations on paper (46–50), thin-layer plates (51, 52), and columns (53–58).



Scheme I

Table I—Analysis of Ascorbic Acid, 100-mg Tablets, after Long-Term Storage at 25°

Lot Number	Storage Time, Months	Assay, % of Claim[a]	Initial Assay, % of Claim[a]	Loss in Potency, %	Diketogulonic Acid, %	Oxalic Acid, %	Dehydroascorbic Acid, %
V-418	103	99	103	4	1	0.5	0.5
KRK-202-66-I	103	104	107	3	2	0.5	0.2
KRK-202-65-III	103	106	110	4	1	0.5	0.2
KRK-202-65-IV	103	106	110	4	1	0.5	0.4
KRK-202-66-II	103	105	111	6	2	1.0	2.4
DMS-289-II	90	104	102	—	0.5	0.5	0.3
Lot 2082	120	111	—	—	—	0.2	0.4
Lot 2694B	240	101	—	—	—	0.4	2.3
Lot 002-0B097A	96	98	—	—	—	—	1.3

[a] Assay by iodometric and 2,6-dichloroindophenol titrations.

EXPERIMENTAL

Stability Tests of Ascorbic Acid Tablets—Samples of commercial ascorbic acid tablets stored for many years at room temperature in closed bottles were assayed for ascorbic acid, dehydroascorbic acid, diketogulonic acid, and oxalic acid.

Commercial ascorbic acid tablets were stored under various test conditions as detailed later. The tablets contained 100 mg of ascorbic acid, dextrins, starch USP, lactose, talc, and magnesium stearate. Tablets were stored at room temperature (25°) and in the refrigerator (5°) in glass and in polyethylene bottles. The bottles were opened daily (five times per week) and returned to closed storage to simulate normal conditions of use. Tablets were also stored in an open dish at 25°.

Assays were run at monthly intervals for ascorbic acid, dehydroascorbic acid, diketogulonic acid, oxalic acid, and moisture, using whole tablets. Scrapings from the tablet surfaces, 15–20% of the total tablet weights, were assayed for intact ascorbic acid.

Analytical Methods—Ascorbic acid was determined by titration with iodine (22, 23) and 2,6-dichloroindophenol (23, 24) and by differential pulse polarography[1]. Dehydroascorbic acid was determined fluorometrically (40). Diketogulonic acid and oxalic acid were determined by TLC[2] (59). In addition to the TLC methods, oxalic acid was determined by a modification[3] of the fluorometric method of Zarembski and Hodgkinson (60). Moisture content of tablets was determined by drying at 105° for 3 hr. The specificity of these analytical methods for determining intact ascorbic acid in the presence of decomposition products was evaluated by assaying an aqueous solution of ascorbic acid at pH 4 which was heated at 45° for varying periods to induce graded levels of ascorbic acid decomposition up to 40%.

IR Spectroscopic Determination of Ascorbic Acid Stability—IR curves were determined for ascorbic acid, dehydroascorbic acid, 2-ketogulonic acid[4], oxalic acid, and tablet placebos to assess the utility of IR measurements as an indicator of the stability of ascorbic acid in tablets as suggested by Wilk (21). All measurements were made using potassium bromide pellets, since no suitable solvent is available for preparing solutions of ascorbic acid and its degradation products that is compatible with the commonly used IR cell (sodium chloride).

RESULTS

Long-Term Storage at Room Temperature—Table I shows the results of assays for ascorbic acid and potential breakdown products in various commercial tablets stored for many years in closed bottles at room temperature. The excellent stability of ascorbic acid in these formulations is evidenced by the fact that all maintained the claimed level for periods of 5 years and longer. The amounts of decomposition products of ascorbic acid found, namely, dehydroascorbic acid, diketogulonic acid, and oxalic acid, when detectable, represented only a very small percentage of the ascorbic acid content.

Short-Term Use Tests at 5 and 25°—The results of these tests for 20 weeks to date are summarized in Table II. Assays of whole tablets showed no measurable loss at 25° in open storage or in amber or polyethylene bottles opened daily five times per week; the retention of ascorbic acid by three assay methods averaged 100% of claim after 3 months. The surface scrapings (15–20% of the tablet weight) showed average retentions of 96.5 and 96% at 5 and 25°, respectively, after 20 weeks. These small losses of 3.5–4% in 15–20% of the tablet weight would be equivalent to losses of 0.7–0.8% in the whole tablets. It is clear that greater losses of ascorbic acid occur at the surface of the tablets, as expected.

The amounts of dehydroascorbic acid measured in these stored tablets after 20 weeks are equivalent to about 3% of the original ascorbic acid content. Dehydroascorbic acid is fully active as ascorbic acid and represents no loss in vitamin activity. No detectable amount of diketogulonic acid could be seen on the thin-layer chromatograms after any storage test. The maximum amount of oxalic acid found in these storage tests is equivalent to about 0.2% of the ascorbic acid content, a trivial amount.

The overall averages of all assays of ascorbic acid in the stability tests listed in Table II by iodometric titration, 2,6-dichloroindophenol, and polarographic measurements are 100, 101, and 100%, respectively. This agreement confirms the utility of all three methods for measuring ascorbic acid in these tablets.

DISCUSSION

Adequacy of Analytical Methods for Ascorbic Acid—The direct titration of ascorbic acid with iodine or 2,6-dichloroindophenol, as per the methods for tablets in the official compendia (23, 24), is eminently suitable for tablets, since neither the decomposition products of ascorbic acid nor the normal excipients in such tablets have reducing activity. Deutsch and Weeks (40) assayed tablets containing ascorbic acid, multivitamins, and multivitamins–minerals by titration with 2,6-dichloroindophenol and *N*-bromosuccinimide, by colorimetry with diazotized *p*-nitroaniline, and by fluorometry after oxidation to dehydroascorbic acid. They found excellent agreement by all four methods.

Differential pulse polarography, a newer technique, showed close agreement to titration with iodine or 2,6-dichloroindophenol in the present study. Although not necessary for simple ascorbic acid tablets, several chromatographic methods are available to provide specific assays for ascorbic acid in the presence of interfering substances (*e.g.*, multivitamin tablets, low-potency foods, and biological samples).

Assays by four different methods of deliberately decomposed solutions of ascorbic acid are summarized in Table III. It is evident from the agreement among results and correlation with specific determinations quantitating the level of degradation products found by the various methods that all four methods measure intact residual ascorbic acid after extensive degradation has occurred. Moreover, the methods designed to determine specific degradation products are capable of doing so when detectable amounts of these materials are present. Wilk's (21) contention that there are no specific assays for ascorbic acid is unfounded.

IR Spectroscopic Determination of Ascorbic Acid Stability—IR curves were determined for pure ascorbic acid, dehydroascorbic acid, diketogulonic acid, oxalic acid, and tablet placebos to assess the utility of IR measurements as an indicator of the stability of ascorbic acid in tablets as proposed by Wilk (21). The spectrum of ascorbic acid does vary from those of the degradation products, as expected, and the band assignments stated in the literature were substantiated (Figs. 1–5). However, a great deal of band overlap is observed with ascorbic

[1] J. B. Johnson, M. J. Frank, J. McCaffrey, and S. H. Rubin, to be published.
[2] Due to the instability of 2,3-diketogulonic acid, 2-ketogulonic acid was used as a reference standard.
[3] M. Osadca and M. Araujo, to be published.
[4] Due to the instability of 2,3-diketogulonic acid, the IR spectrum of 2-ketogulonic acid, which is of similar structure, is shown.

Table II—Analysis of Ascorbic Acid, 100-mg Tablets (Lot JG-4969-58-2A)

Storage Conditions	Assay, % of Claim: Iodometric Total	Iodometric Surface	2,6-Dichloroindophenol Total	2,6-Dichloroindophenol Surface	Polarographic Total	Polarographic Surface	Percent of Moisture Content	Average Weight, mg/Tablet	Degradation Products[a] (Fluorometric Analysis): Dehydroascorbic Acid, %	Oxalic Acid, %
Initial	102	97	103	96	100	97	1.23	401	—	—
4 weeks/5°										
Amber glass bottle	99	102	103	90	101	97	1.05	401	—	—
Polyethylene bottle	104	103	103	100	100	99	1.33	401	—	—
4 weeks/25°										
Amber glass bottle	102	98	100	99	99	103	1.20	404	1.3	0.04
Polyethylene bottle	105	100	98	101	99	98	1.20	398	1.1	N.D.[b]
Open dish	98	93	99	98	101	100	1.37	396	1.2	0.03
8 weeks/5°										
Amber glass bottle	104	102	99	94	100	92	1.21	403	3.1	N.D.
Polyethylene bottle	97	94	100	94	100	96	1.25	399	3.5	N.D.
8 weeks/25°										
Amber glass bottle	101	92	102	93	98	94	1.15	403	2.1	0.07
Polyethylene bottle	99	89	100	90	100	95	1.20	396	2.4	0.04
Open dish	100	85	101	91	98	91	1.45	397	3.1	0.03
12 weeks/5°										
Amber glass bottle	98	92	100	92	97	95	1.30	402	3.3	N.D.
Polyethylene bottle	98	92	99	95	98	98	1.21	400	2.9	N.D.
12 weeks/25°										
Amber glass bottle	101	92	100	96	100	95	1.16	400	3.1	0.03
Polyethylene bottle	98	97	101	96	103	94	1.22	398	2.8	N.D.
Open dish	100	95	101	93	103	95	1.16	397	2.9	N.D.
16 weeks/5°										
Amber glass bottle	97	94	98	95	99	92	1.16	403	3.6	—
Polyethylene bottle	103	100	103	101	103	98	1.38	400	3.5	—
16 weeks/25°										
Amber glass bottle	100	93	102	93	102	95	1.00	405	3.7	—
Polyethylene bottle	103	95	99	95	100	95	1.10	398	2.8	—
Open dish	98	89	99	91	100	88	0.94	395	4.6	—
20 weeks/5°										
Amber glass bottle	96	96	95	95	100	97	1.47	400	5.4	—
Polyethylene bottle	99	99	102	96	101	96	1.56	400	4.5	—
20 weeks/25°										
Amber glass bottle	100	96	100	94	100	96	1.16	401	5.8	—
Polyethylene bottle	98	98	99	97	99	98	1.27	396	5.8	—
Open dish	98	95	98	96	99	96	1.28	396	—	—

[a] No degradation products were found using TLC. Limits of detection were 0.02% for diketogulonic acid and 0.07% for oxalic acid. [b] N.D. = none detected.

acid, dehydroascorbic acid, diketogulonic acid, and oxalic acid, as well as a large baseline displacement from the placebo. The combined effect of overlapping bands, placebo interference, and the availability of only imprecise methods for sample preparation make the use of IR impractical as a quantitative tool for ascorbic acid.

Stability of Ascorbic Acid in Tablets—The data in Table I show clearly that commercial ascorbic acid tablets stored at room temperature in closed bottles retain full label potency over a shelflife period of many years. In the use tests reported in Table II, the stability of ascorbic acid in the 100-mg tablets was excellent throughout the 3-month storage period involving intermittent brief exposure at both the 5 and 25° storage conditions and in open storage at 25° for the entire period. The relative humidity of the laboratory atmosphere to which these tablets were exposed ranged from 55 to 65%.

In 3-week storage tests at 45° of simple powder mixtures of ascorbic acid with tablet excipients, it was demonstrated (61) that moisture content is a critical factor determining ascorbic acid stability. At moisture levels below 2%, the stability of ascorbic acid was excellent, but progressively higher levels of moisture caused increasing but limited losses of the vitamin. The data in Tables I and II show that ascorbic acid tablets manufactured with proper control of moisture content have excellent stability. The moisture content of the tablets in Table II was low initially and remained low throughout the exposure tests, indicating that the compressed tablets are strongly resistant to moisture uptake under the stated conditions.

Significance of Dehydroascorbic Acid Formation—Wilk (21) implied that dehydroascorbic acid formed by oxidation of ascorbic acid represented a potentially harmful substance due to alleged diabetogenic activity. Actually, the diabetogenic activity referred to was that reported by Patterson (62) following *massive, intravenous* in-

Table III—Validation of Assay Procedures (Solution: 100 mg/ml, pH 4.0)

Incubation Conditions	Iodometric (23) Titration, % of Claim	2,6-Dichloroindophenol (23) Titration, % of Claim	Colorimetric (33) Assay, % of Claim	Polarographic Assay, % of Claim	TLC Determination: Diketogulonic Acid, mg/ml	TLC Determination: Oxalic Acid, mg/ml	Fluorometric (40) Determination of Dehydroascorbic Acid, mg/ml	pH
Initial	100	100	100	100	N.D.[a]	N.D.	N.D.	4.0
3 days/45°	85	86	86	86	6	5	0.2	—
7 days/45°	71	72	72	71	10	15	3.6	—
11 days/45°	62	61	60	62	10	20	5.1	4.3

[a] N.D. = none detected.

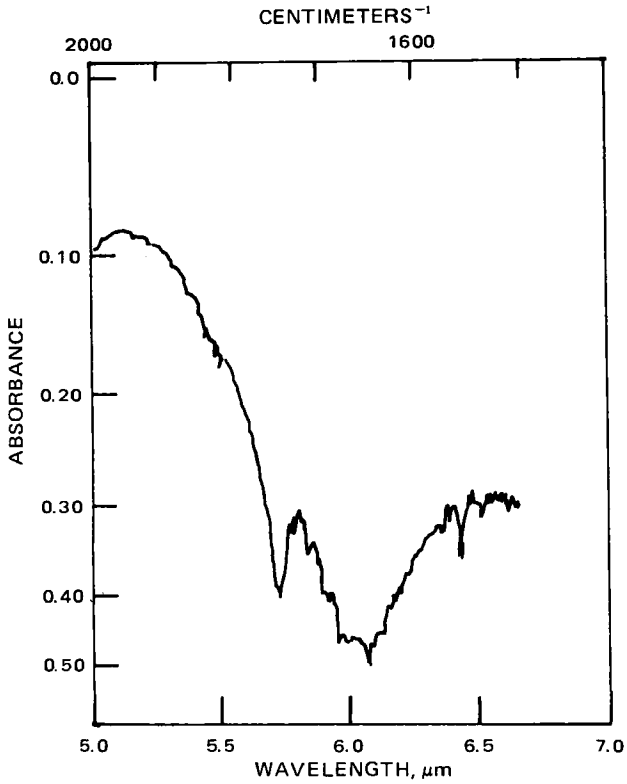


Figure 1—*IR spectrum of ascorbic acid standard (USP), 3.0 mg/350 mg (potassium bromide pellet).*

jection of dehydroascorbic acid into rats. The doses involved were close to lethal and were, in fact, lethal to many animals.

No diabetogenic activity of dehydroascorbic acid has been reported for oral dosage. On the contrary, a number of reports show that orally administered dehydroascorbic acid is quantitatively equal to ascorbic

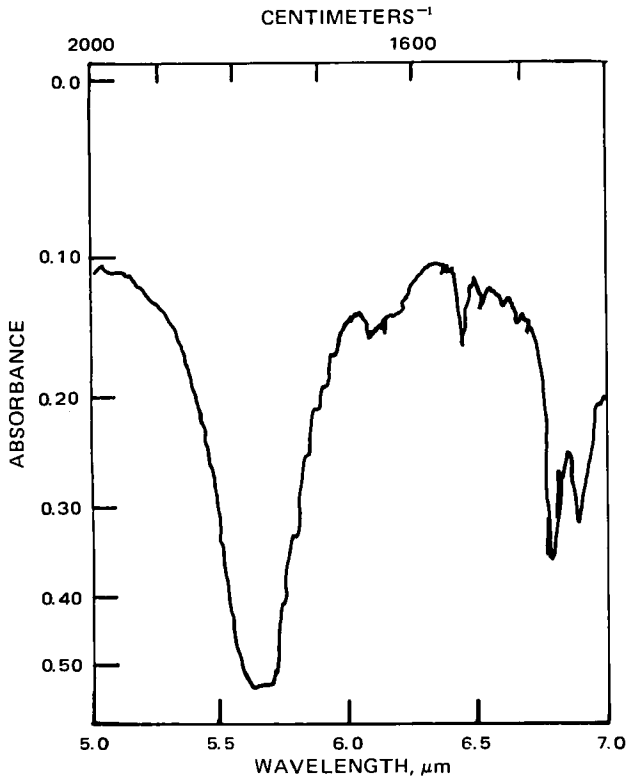


Figure 2—*IR spectrum of dehydroascorbic acid, 3.0 mg/350 mg (potassium bromide pellet).*

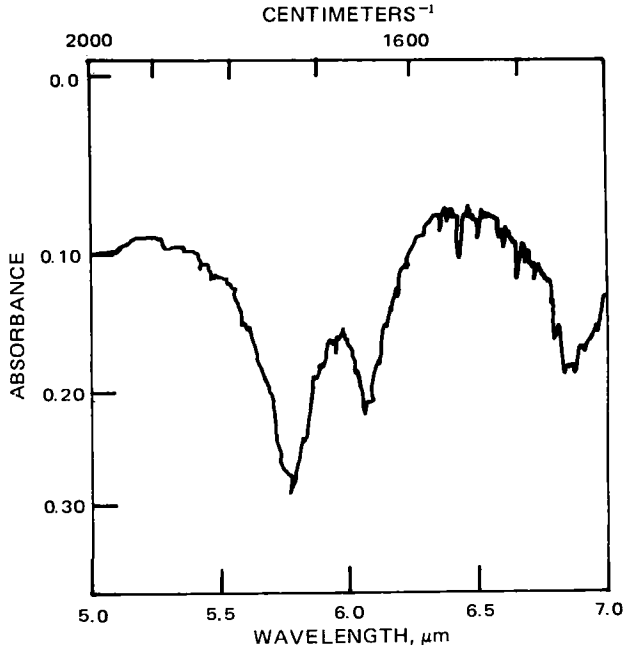


Figure 3—*IR spectrum of 2-ketogulonic acid, 3.0 mg/350 mg (potassium bromide pellet).*

acid for humans. This equivalence was demonstrated by measuring urinary excretion of ascorbic acid after comparable doses of dehydroascorbic acid and ascorbic acid in the 300–600-mg range (63, 64). Comparable blood levels and urinary excretion of ascorbic acid were found in young women following consumption of 65 mg of ascorbic acid from orange juice or 65 mg of dehydroascorbic acid from activated charcoal-treated orange juice (65). Oral intake of equal amounts of ascorbic acid or dehydroascorbic acid resulted in equivalent increases in both ascorbic acid and dehydroascorbic acid levels in the blood of human subjects (66, 67). Thus, the biological utility of doses of dehydroascorbic acid has been well documented. The small quantities of dehydroascorbic acid that may occur in aged ascorbic acid tablets represent full vitamin activity and present no dietary hazard.

Significance of Oxalic Acid Formation—Although Wilk (21) did not measure oxalic acid in aged ascorbic acid tablets, the possible occurrence of oxalic acid in such tablets was cited by him as being potentially harmful to the consumer. The actual amounts of oxalic acid found in aged tablets in the present study (Tables I and II) were small: only 1.0 mg or less in a 100-mg tablet.

These amounts of oxalic acid are negligible compared to amounts

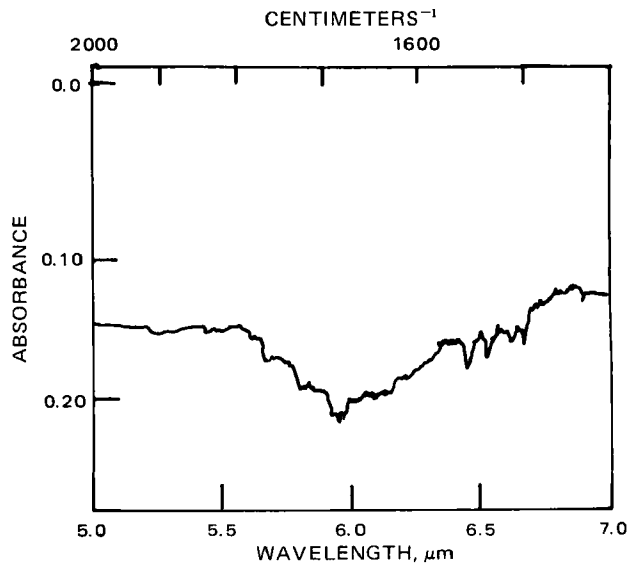


Figure 4—*IR spectrum of oxalic acid, 5.0 mg/350 mg (potassium bromide pellet).*

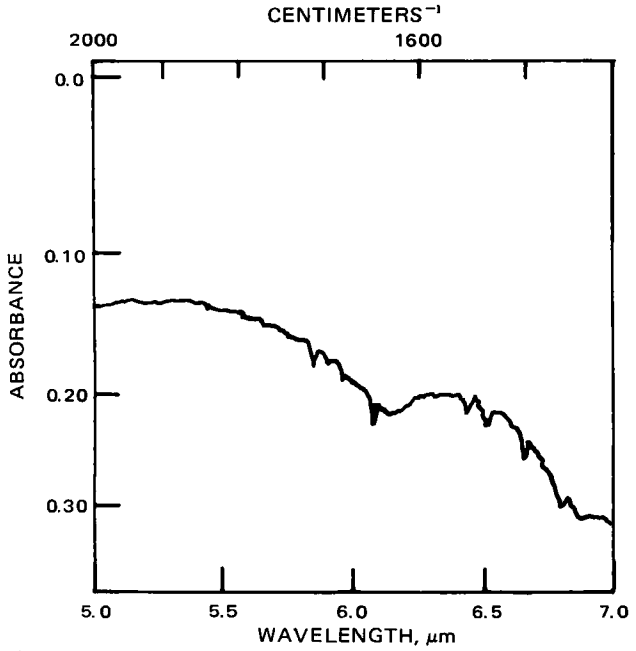


Figure 5—*IR spectrum of tablet placebo, 3.0 mg/350 mg (potassium bromide pellet).*

normally consumed by humans in the diet. For example, 82 and 150 mg of oxalic acid/100 g were found in two composite meals (68), and as much as 850–980 mg of anhydrous oxalic acid was found in 10 daily English diets from which high oxalate foods such as rhubarb and spinach were excluded (69). Obviously, the ingestion of even a number of aged tablets daily will cause practically no increase in the intake of oxalic acid.

The metabolic breakdown of ascorbic acid in human subjects has been studied (70). In a group of 10 subjects, the average excretion of a 1-g dose of ascorbic acid was approximately 65% as unchanged ascorbic acid, 6% as dehydroascorbic acid, and 2.3% as diketogulonic acid. After 90 days of dosing with 1 g of ascorbic acid/day or 2 g/day for 180 days, average increases in oxalic acid excretion were equivalent to less than 0.5% of the daily dose of ascorbic acid and were not statistically significant.

Lactose Intake from Ascorbic Acid Tablets—Wilk (21) cited the presence of lactose, commonly used as an excipient in ascorbic acid tablets, as a problem for lactose-intolerant individuals ingesting large numbers of such tablets, *e.g.*, 40 tablets to provide 4 g of vitamin. However, it is extremely unlikely that anyone wishing to take such a quantity would use 100-mg tablets, since 500-mg tablets are readily available and much more convenient. With the higher potency tablets, the intake of excipients is proportionately much less.

Even the theoretical consumption of 40 tablets containing 100 mg of ascorbic acid each would provide, with usual formulations, not more than 3.6 g of lactose. This quantity is relatively small compared to approximately 12 g in a glass of milk, which is tolerated by most people who are sensitive to a lactose tolerance test of 53–100 g.

Sodium Content of Chewable Ascorbic Acid Tablets—The question has been raised whether the amount of sodium from sodium ascorbate in chewable tablets is sufficient to be of concern to patients on a low-sodium diet. For taste reasons, a mixture of sodium ascorbate and ascorbic acid is sometimes used in such tablets. A typical formulation contains 60% of the ascorbic acid as the sodium salt, resulting in a sodium content of about 20 mg/tablet. The average daily dietary intake of sodium chloride of 6–18 g is equivalent to 120–360 times the amount of sodium in such a chewable vitamin tablet. Nonchewable tablets usually contain ascorbic acid in the free acid form only and contribute no sodium to the diet.

CONCLUSIONS

Contrary to allegations by Wilk (21), ascorbic acid tablets stored in closed bottles retain their claimed vitamin potency for 5 years or longer at room temperature. Storage of 100-mg tablets in an open dish at 25° or daily opening of glass or polyethylene bottles stored at room temperature or in the refrigerator for 20 weeks to date caused from 0 to 2% loss of ascorbic acid; slight losses occurred close to the surface of the tablets.

Analytical methods with high specificity are available, and have been for years, for determining the stability of ascorbic acid; IR spectroscopy is not suitable for quantitative assessment of ascorbic acid breakdown in tablets.

Very small amounts of dehydroascorbic acid are found in aged tablets. This oxidized form of the vitamin is fully active as ascorbic acid and is not diabetogenic.

Little or no oxalic acid has been found in aged tablets; the minute quantities found are insignificant in relation to normal levels in the diet. Similarly, tablet excipients such as lactose and sodium from sodium ascorbate are present in relatively small quantities, compared to the lactose content of milk and the normal sodium content of the diet, respectively.

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Combined Assays for Vitamins A, D (Ergocalciferol), and E in Multivitamin Preparations with Separation by Reversed-Phase Partition Chromatography

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Abstract □ A rapid method was developed whereby vitamins A, D (ergocalciferol), and E in multivitamin products are measured in a common assay sample. The method depends on reversed-phase partition chromatography by which the vitamin alcohols are eluted in separate fractions from a column. Vitamins A and E are then determined by their UV absorption, while vitamin D is measured colorimetrically with an antimony trichloride reagent. The column consists of diatomaceous earth impregnated with dimethyl polysiloxane, with *n*-heptane as the immobile solvent, and 90% methanol followed by 95% methanol as the mobile solvents. Vitamins A and D elute in that order in the 90% methanol, and finally vitamin E elutes in the 95% methanol fraction. The method is widely applicable to various types of multivitamin and vitamin–mineral products including oil-based, water-based, and dry formulations.

Keyphrases □ Vitamins—combined analyses, vitamins A, D (ergocalciferol), and E in multivitamin preparations, reversed-phase partition chromatography □ Chromatography, reversed-phase partition—combined analyses, vitamins A, D, and E in multivitamin preparations □ Multivitamin preparations—combined analyses, vitamins A, D, and E, reversed-phase partition chromatography

The USP (1) provides assays for vitamins A and E in multivitamin preparations which utilize a common assay sample. However, duplication of procedure exists in that a similar assay step, depending on the selective degradation of vitamin A in the presence of vitamin E, is subsequently carried out in each of the two determinations. Duplication of assay steps is also involved with respect to the USP assay for vitamin D (ergocalciferol) (1). The procedure is similar to that for vitamins A and E through the initial steps of alkaline hydrolysis and extraction, but another sample is nevertheless taken and the complete analysis is carried out separately.

The USP vitamin D assay also is subject to the criticism that vitamin E, if present, carries through the analysis, causing high results for vitamin D. To correct for such interference, procedures have been proposed for the removal of vitamin E by adsorption on a column (2, 3); but this additional step lengthens what is already a rather long and tedious assay. Perhaps the main disadvantage is that the vitamin E fraction is lost, so a completely separate determination must be undertaken for vitamin E.

Incorporated in the USP vitamin D assay is a column step for the separation of vitamin D from vitamin A. This step depends on "conventional" partition chromatography; *i.e.*, the more polar solvent is the stationary phase and the less polar is the mobile phase. Conceivably such a system might also provide a separation between vitamins D and E, with vitamin E eluting first. The problem is that vitamins D and E both elute close to the solvent front, so their bands tend to overlap and a complete separation is difficult.

The reversed-phase partition column has been found