Ascorbic Acid, Vitamin C

Ascorbic acid is commonly recognized as an antioxidant, i.e. the compound oxidizes easily. Therefore, the degradation that occurs during storage of aqueous solutions of this compound is also known to be of oxidative nature.

L-ascorbic acid is found in equilibrium with its oxidative degradation product, L-dehydroascorbic acid, which is used in the body in the same way as ascorbic acid. L-dehydroascorbic acid has its maximum stability at room temperature at pH 2-3 and it will at pH values above 7 degrade irreversibly during formation of 2,3-diketogulonic acid which shows no vitamin C activity. Finally, at high pH values 2,3-diketogulonic acid can degrade to threonic, xylonic and oxalic acid. Therefore, at pH values above 7 a reaction will occur leading to a net degradation of the ascorbic acid causing a formation of these degradation products.

Lloyd et al. (1987) have by means of HPLC examined the degradation of L-ascorbic acid and L-dehydroascorbic acid in solution and have determined the presence of the final degradation products, threonic and oxalic acid, but not the intermediate product 2,3-diketogulonic acid. Since the applied method is able to detect the presence of this compound, it is suggested that, apart from the above-mentioned mechanism, an alternative degradation route should be found, which will lead to the same end products.

Aqueous ascorbic acid solutions are the stablest at weakly acidic to neutral pH values (5-7). Hüttenrauch (1968) has determined the half life of aqueous ascorbic acid solutions at different pH values and has found that the half life is the largest in a neutral environment, in which the compound exists as monoanion.

<table>
<thead>
<tr>
<th>pH value</th>
<th>Half life at 25°, weeks</th>
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<tbody>
<tr>
<td>5.5</td>
<td>1414</td>
</tr>
<tr>
<td>6.5</td>
<td>2875</td>
</tr>
<tr>
<td>7.2</td>
<td>4175</td>
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</tbody>
</table>
At pH values under approx. 5.5 and above approx. 9, respectively, more than about 10% of the compound is found as free acid and as divalent anion, both of which are much less stable than the monoanion form.

Biotin and its stability is not given much attention in the chemical and pharmaceutical literature. The handbooks describe that the pure compound is stable to air and heat. Biotin is not sensitive to weak oxidizing agents (air), reducing agents or visible light. Strong oxidizing agents, UV light and acids have negative effects on the stability of the compound. It has not been possible to find descriptions of the degradation compounds formed during long term storage of biotin even though we have searched in the international databases. It must be concluded that this aspect of the chemistry of biotin has not been examined.

Calcium-D-pantothenate is less stable than the majority of the vitamin B group. It is necessary to keep the compound dry in a closed container if degradation is to be avoided. It is generally known that a solution of calcium-D-pantothenate will hydrolyze to β-alanin and 2,4-dihydroxy-3,3-dimethyl butyric acid, the latter of which might be on lactone form depending on pH. Frank et al. (1973) made mixtures of calciumpantothenate and some acidic compounds, e.g. ascorbic acid, and tested their stability. The group observed that even under these dry conditions, calciumpantothenate is degraded, in fact the content of β-alanin is increasing with time. In parallel to what has been proposed in earlier references, the group proposes that the compound is degraded by a general acid hydrolysis, irrespective of the experiment being performed in rather dry conditions.

Cholecalciferol, Vitamin D₃

A review of the available literature on degradation of vitamin D during storage has not led to any literature specifically about degradation products of this compound. There are some articles that describe the stability of vitamin D under various conditions. However, the authors do not mention which compounds are formed, when vitamin D is degraded.
Our supplier of cholecalciferol, Hoffmann-La Roche, states that, “The nature of cholecalciferol’s degradation products is unknown. Therefore, the stability is only examined by carrying out the assay for vitamin D₃ after storage under the chosen conditions”. (Hoffmann-La Roche 1989).

**Cyanocobalamin (Vitamin B₁₂)** is one of the most sensitive and thus least stable vitamins. It is affected by pH, light, heat, humidity and oxidizing compounds and in liquid drug forms chemical interactions with other vitamins, mainly ascorbic acid and thiamine, are pronounced. Cyanocobalamin undergoes a photolytic degradation leading to the formation of hydroxycobalamin. Hydroxycobalamin has the same biological activity as cyanocobalamin but prolonged exposure to light will cause a further degradation to non-active compounds. Even though the reactivity of cyanocobalamin is less pronounced in dry form, it is still recommended to stabilize the compound by a dry dilution in which the substance is triturated with mannitol or galactomannan. The first solution is used in the present preparation.

**Folic acid (Vitamin-M)** is composed of p-aminobenzoic acid alkylated to a molecule of 2-amino-4-hydroxy-pteridine-6-carboxylic acid which also is acylated to L-glutamic acid. The bond between the amino group in p-aminobenzoic acid and 6-methyl in the pteridin skeleton is the most unstable bond in the molecule. Oxidative cleavage of this bond – which might be caused by oxygen and light or by potassium permanganate in alkaline solution – will lead to the formation of 2-amino-4-hydroxypteridin-6-carboxylic acid and p-aminobenzoyl glutamic acid. Analogously, a hydrolysis will lead to the formation of p-aminobenzoyl glutamic acid and 2-amino-4-hydroxypteridin-6-carbaldehyde. Another aspect of the stability of folic acid is its tendency to undergo chemical interactions with other vitamins, especially thiamine and riboflavin. Although studies on solid drug forms are not available, studies on liquid formulations (Biamonte & Schneller 1951, Darnula & Colah 1972) show that folic acid is not stable in the presence of thiamine or riboflavin, not even at the pH-values where folic acid is undissolved. The authors do not mention which compounds are formed when folic acid is degraded. A possible way of overcoming the interactions with other vitamins is to add folic acid in the tablet coating or to produce multi-layer
tablets - however, this possibility is not used in the present formulation.

**Retinyl palmitate, vitamin A palmitate**

BASF Danmark A/S states that the presence of the two antioxidants, ascorbic acid and tocopherol, will contribute considerably to the prevention of degradation of the vitamin A palmitate. Oxidative degradation of vitamin A palmitate may cause the formation of a countless number of low-molecular compounds, which cannot all be identified. The main part of these will, however, probably be isoprenoid carbon compounds, such as β-ionone.

Thermal degradation of vitamin A palmitate, which is an all-trans configuration, will at first lead to the formation of different cis-configurations, which may later dimerize. Isomerization will occur as well if the pH value falls below 4.5 (De Ritter 1977).

**Riboflavin (B_2-Vitamin)** is relatively stable in solid form. In dissolved form the compound is more stable in neutral or acid pH while an alkaline environment and exposure to light will cause the compound to degrade to lumiflavin. A similar process leads to formation of lumichrome from acidic or neutral solutions.

Because of the sensibility to light, all analytical work is prescribed to be performed under subdued light or in dark glass.

**Pyridoxine hydrochloride (B_6-Vitamin)** is generally recognized as a very stable compound as light is the only factor which will have a deteriorating effect on the stability of the compound. The sensitivity to light is more pronounced when the compound is dissolved, and hence analytical work is prescribed to be performed under subdued light or in dark glass. Puech et al. (1981) has examined tablets with vitamin B_6 by means of TLC. The tablets were stored at 23, 27 and 50°C and even at the highest temperature 98% of the content was still present when the experiment was terminated after 18 months. The authors mention that they did not observe any spots
corresponding to degradation products on any of the TLC plates.

**Nicotinamide (B₃-Vitamin)** is one of the most stable vitamins. The compound is described as stable when dry but affected by prolonged exposure to humidity and light. Cascioli et al. (1971) determined the rate constants for degradation of the B vitamins in granulates kept at elevated temperatures. The authors concluded that nicotinamide is stable as no degradation was observed after 15 days at 65°C.

**References**


Lloyd et al., Quantitative Reversed Phase HPLC Analysis of L-Ascorbic Acid (Vitamin C) and Identification of its Degradation Products. Chromatographia 24, 371-376.