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Determination of vitamin C in effervescent tablets containing other vitamins together with trace elements

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Abstract

A simple, rapid method is reported for the determination of vitamin C in effervescent tablets containing other vitamins and several trace elements such as Mg(II), Zn(II), Fe(II), Mn(II), Cu(II) and Mo(VI). The procedure was developed on the basis of the bromate ion–iodide ion–ascorbic acid clock reaction (Landolt reaction). Interference effects of air oxygen and metal ions and the role of pH are discussed in detail.

Keywords: Effervescent tablets; Landolt reaction; Vitamin C

1. Introduction

Vitamin C is one of the most common natural or artificially enriched ingredients in foods and beverages. It is readily oxidized by atmospheric oxygen and/or decomposes under a variety of conditions, in particular in the presence of catalytic impurities. Therefore, the concentration of this compound is an excellent indicator of product quality, stability and uniformity during the manufacturing process or storage.

Recently a fast chronometric method was developed to determine vitamin C in cottage-cheese desserts [1]. This procedure offers a simple, efficient and low-cost alternative to standard determinations of ascorbic acid. The method is based on the well known bromatie ion–iodide ion–ascorbic acid reaction [2]:

$$BrO_3^- + 6I^- + 6H^+ \xrightarrow{\text{slow}} Br^- + 3I_2 + 3H_2O \quad (1)$$

$$H_2A + I_2 \xrightarrow{\text{fast}} A + 2H^+ + 2I^- \quad (2)$$

In these reactions, iodine, produced in the first step, is immediately reduced by conversion of ascorbic acid (H_2A) to dehydroascorbic acid (A). When the oxidant is added in excess, the total consumption of ascorbic acid is indicated by a sudden colour change due to iodine formation. The Landolt time (t_L), i.e. the time lapse between mixing the reagents and the observed colour change, is given as follows:

$$t_L = \frac{[H_2A]}{3k[BrO_3^-][I^-][H^+]^2} \quad (3)$$

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where the denominator is the appropriate rate law for reaction 1. According to earlier studies $k = 44$ dm^9 mol^{-3} s^{-1} [3]. The Landolt time can be conveniently controlled by carefully selecting the concentrations of the reactants and the pH.

In real samples, possible degradation of ascorbic acid may significantly corrupt the result of this simple analytical procedure. The presence of inhomogeneities, such as in the case of milk products, may complicate matters further. In the present study, the above method was adopted to determine vitamin C in the following roborant effervescent products: Béres Vitalin and Béres Vitakid (Béres, Budapest, Hungary), Plusssz Multivitamin, Plusssz C vitamin and Plusssz Vas + C (Pharmavit, Veresegyház, Hungary) and Ca-C 1000 Sandoz (Alkaloida, Tiszavasvári, Hungary). The compositions of these products are listed in Table 1. It is discussed in detail how various components affect determination of vitamin C in these tablets.

2. Experimental

All chemicals were of analytical-reagent grade (Reanal, Budapest, Hungary). Doubly distilled water, de-aerated by purging with nitrogen, was used for the preparation of stock solutions and reaction mixtures. Stock solutions of L-ascorbic acid and starch solutions were prepared freshly. The tablets were finely pulverized and homogenized prior to use. During the analysis, the solutions were continuously purged with nitrogen.

The samples were analysed in two different ways, by either a direct or a standard addition method. For the direct measurements the reaction mixtures were prepared with the following composition: 2 cm^3 of sample solution in which the H_2A concentration was about 8×10^{-4} mol dm^{-3}, 4 cm^3 of 0.05 mol dm^{-3} $KBrO_3$, 1 cm^3 of 0.5% starch solution, 4 cm^3 of 0.05 mol dm^{-3} KI and 8 cm^3 of water. The clock reaction was triggered by injecting 1 cm^3 of 0.4 mol dm^{-3} HCl into the well stirred reaction mixture. The Landolt time was measured with a digital stop watch to ± 0.01 s. The concentration was calculated on the basis of t_L vs. $[H_2A]$ calibration curves obtained with standard ascorbic acid solutions. In the standard addition method the ascorbic acid concentration of each sample was determined on the basis of a series of six kinetic runs. The reaction mixtures were prepared as in the direct experiments. In a series, various amounts of standard ascorbic acid solution were added to the individual reaction mixtures, each containing the same amount of sample. The total ascorbic acid concentration (sample + standard solution) was in the same region as in the direct experiments (8×10^{-5}–4×10^{-4} mol dm^{-3}). The ascorbic acid concentration of the sample was obtained by plotting t_L vs. the added ascorbic acid concentration. It should be emphasized that this procedure differs from the usual standard addition methods in that the concentration of the added analyte is varied by using otherwise identical reaction mixtures instead of using the same sample solution. Nevertheless, the basic principle, i.e. using the matrix of the sample for the determination, is the same as in the classical standard addition technique.

3. Results and discussion

The products investigated in the present study are used for the treatment of vitamin and/or trace element deficiencies. For these commercially available roborants only the composition and quantity of the active substance (vitamins, metal, ions, etc.) are given on the packing material, with only qualitative information for the rest of the ingredients. In Table 1, the tablets are grouped on the basis of their composition. It is noteworthy that a given manufacturer uses almost the same choice of components for all of its products.

The tablets in the first group contain base components (such as hydrogencarbonate, organic acid, sweetening agents, flavours and different ingredients necessary for tableting), vitamins (including vitamin C) and trace elements. Ca-C 1000 Sandoz is placed in the third group. Only limited information is provided for its composition. It is known, however, that large amounts of vitamin C and calcium salts are contained in this product.

Table 1
Composition of tablets

(A) First group (Béres family) Ingredients	Béres Vitalin (mg/4.6 g tablet)	Béres Vitakid (mg/3.5 g tablet)
Vitamin C	60	35
Niacin	17	7.5
Vitamin E	–	3.8
Ca D-pantothenate	7.64	2.61
Vitamin B_6	2.19	0.8
Vitamin B_2	1.5	0.65
Vitamin B_1	1.46	0.6
Mg(II)	31	12
Fe(II)	4	2
Zn(II)	10	4
Cu(II)	1.5	0.6
Mn(II)	1	0.5
Mo(VI)	0.075	0.03
Se	0.02	0.01
Succinic acid	50	20
Glycine	30	15
Aspartame	75	65
D-Mannitol	+	+
D,L-Hydroxysuccinic acid	+	+
Potassium hydrogencarbonate	+	+
Polyethylene glycol	+	–
Lemon flavour	+	–
Orange flavor	–	+
Food dyestuff	–	+

(B) Second group (Plusssz family) Ingredients	Multivitamin (mg/4g tablet)	C vitamin (mg/4 g tablet)	Vas+C (mg/4.5 g tablet)
Vitamin C	60	75	75
Nicotinamide	11	–	–
Ca D-pantothenate	8	–	–
Vitamin E	7	–	–
Vitamin B_2	11	0.5	–
Vitamin B_6	1.4	–	–
Vitamin B_1	1.1	–	–
Vitamin B_{12}	0.002	–	–
Iron(II) gluconate	–	–	35
Aspartame	50	50	40
Citric acid	+	+	+
Saccharose	+	+	+
Sodium hydrogencarbonate	+	+	+
Polyethylene glycol	+	–	–
Orange flavour	+	–	–
Pineapple flavour	–	–	+
Tricalcium phosphate	–	–	+
Food dyestuff	–	–	+

Table 1 (continued)
Composition of tablets

(C) Third group Ingredients	Ca-C 1000 Sandoz (mg/7 g tablet)
Vitamin C	1000
Calcium lactate gluconate	1000
Calcium carbonate	327
Orange flavour	+

3.1. Optimum concentration of reagents

The concentration dependence of the Landolt time is given by Eq. 3. It follows that t_L falls in a conveniently measurable range (9–76 s) if the concentrations are as follows [3]: $[H_2A] = 5 \times 10^{-5}$–$4 \times 10^{-4}$ mol dm^{-3}, $[I^-] = [BrO_3^-] = 0.01$ mol dm^{-3} and $[H^+]$ (added as HCl) = 0.02 mol dm^{-3}. Under these conditions, the error of the measurements, tested with ascorbic acid stock solution, is less than 1%.

3.2. Interference effects

There are several factors which may significantly alter the rate of the Landolt reaction and eventually interfere with the analytical procedure. These effects should be minimized or, if it is possible, eliminated. The following artifacts may alter the analytical procedure: oxidation of ascorbic acid by atmospheric oxygen; substances which consume any of the reagents (these compounds may occur as impurities); potential catalysts of the Landolt reaction; changes in pH. In the last respect it should be noted that t_L is very sensitive to any change in the pH, since it is inversely proportional to the square of the hydrogen ion concentration.

Preliminary experiments clearly indicated certain inconsistencies in the analysis, which were attributed to the above interferences. In direct measurements, higher than the nominal amounts of vitamin C were determined in all cases, the assays ranging from 110 to 179% of the expected values. Moreover, with Vitakid a linear relationship was found between the measured ascorbic acid concentration and the amount of the sample. In another series, the standard addition technique gave lower concentrations than those obtained from the direct measurements. The measured ascorbic acid content ranged from 80 to 113% of the nominal value.

3.3. Role of dioxygen

In the presence of air, ascorbic acid readily oxidized to dehydroascorbic acid above pH 4. The process is catalysed by metal ions and riboflavin [4]. Since the effervescent tablets contain these components, and the pH of the samples is generally higher than 4, the stability of the sample solutions was tested. As expected, the stability of ascorbic acid in the analysed products varied significantly (Table 2). In the second group only a slight degradation (less than 1%) was observed within the studied time interval. In the freshly prepared solution of Ca-C 1000 Sandoz about 3% degradation was detected after 1 h.

Considerably greater degradation was observed for the members of the first group, especially Vitakid. These tablets differ from the second group in their metal content only. The ca. 30%

Table 2
Stability of sample solutions

Tablets	Time (min)	Degradation (%)
Béres Vitalin	105	5.3
Béres Vitakid	90	30.0
Plusssz Multivitamin	95	0.9
Plusssz C vitamin	63	0.8
Plusssz Vas+C	64	0.5
Ca-C 1000 Sandoz	63	3.2

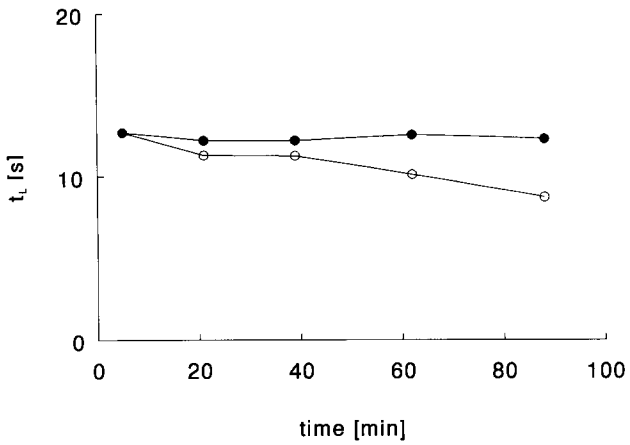

Fig. 1. Stability of Vitakid sample solutions: (●) purging the samples with nitrogen; (○) without the removal of oxygen.

degradation of Vitakid compared with 5.3% degradation of Vitalin also indicates the importance of the actual composition. In Vitakid the concentration ratios of ascorbic acid to metal ions and ascorbic acid to riboflavin are higher than in Vitalin. This is expected to enhance the stability of vitamin C in Vitakid. Therefore, the noted difference in the instability of these products is most likely due to vitamin E, which is a component of Vitakid only.

The degradation of Vitakid samples was studied as follows. A portion of a Vitakid solution was exposed to atmospheric oxygen and aliquots were withdrawn and analysed for vitamin C from time to time. Another portion of the same sample was purged continuously with nitrogen and used as a reference. As shown in Fig. 1, the ascorbic acid concentrations obtained in the absence of oxygen were practically the same over an extended period of time. The results did not show any degradation, and the RSD was 1.8% for five measurements. According to the direct measurements, in the other solution definite degradation was observed. After 88 min, 30% of the original ascorbic acid was oxidized to dehydroascorbic acid. (By using the standard addition technique less degradation, ca. 23% was observed).

These experiments clearly show that in order to prevent the degradation of the analyte, the samples should be prepared and processed in thoroughly de-aerated solutions.

3.4. Role of metal ions

Transition metal ions may interfere with the BrO_3^-–I^-–ascorbic acid Landolt reaction in several ways. Metal ions may oxidize vitamin C [5–8], affect its autoxidation [5,8,9] or, under certain conditions, oxidize I^- to I_2 [10]. Furthermore, the Landolt reaction itself is catalysed by various transition metal ions [3,11–13]. All of these effects shorten the clock period. It should be emphasized that with the removal of atmospheric oxygen the autoxidation reactions are eliminated.

The added metal ion content is available for the first group only (Table 1). From these ions, Mg^{2+} and Zn^{2+} are inactive and have no effect on the Landolt time. On the basis of previous literature, iron(II) is involved in complexation reactions with L-ascorbic acid [14]. During the analytical procedure, in the presence of the strong oxidant BrO_3^-, iron(II) species may easily be oxidized to iron(III). In turn, iron(III) may lead to a variety of interfering side reactions and catalytic cycles [6,15]. However, such interferences were not observed with the analysed samples.

Molybdenum(VI) is a well known catalyst of the BrO_3^-–I^-–ascorbic acid Landolt reaction [3,11,12]. In the presence of Mo(VI) the following rate equation was confirmed:

$$[H_2A]_0/t_L = 3k[BrO_3^-][I^-][H^+]^2 + 3k_2[BrO_3^-][I^-][H^+]^2[Mo] \quad (4)$$

where $k_2 = 4.3 \times 10^6$ dm^{12} mol^{-4} s^{-1} [3]. The molybdenum concentration is about 2.0×10^{-7} mol dm^{-3}, in Vitalin reaction mixtures and slightly lower, 1.6×10^{-7} mol dm^{-3}, in Vitakid solutions. Provided that molybdenum also shows catalytic activity in the given matrix, it would reduce the Landolt time by about 0.7% (Vitalin) and 0.5% (Vitakid) in the direct experiments. This artifact is eliminated when the standard addition method is used.

Table 3
Results for different Vitakid base granules-added ascorbic acid samples

Granule	Ascorbic added (mg)	Direct measurements		Standard addition	
		Ascorbic acid (mg)	Recovery (%)	Ascorbic acid (mg)	Recovery (%)
I	35.4	36.55	103.2	37.03	104.6
II	35.1	33.92	96.6	37.30	106.3
III	35.0	73.24	209.3	37.51	107.2
IV	36.1	31.14	86.3	30.34	84.0
V	36.5	36.70	100.5	37.79	103.5

Copper(II) may oxidize both I^- and ascorbic acid [5,16]. As a result of these reactions, the Cu content would directly or indirectly (see also Eq. 2) remove 3.5% and 2.3% ascorbic acid in the Vitalin and Vitakid experiments, respectively. This would justify a 2–4% negative error in the Landolt time. Preliminary experiments indicated a more complex pattern. Therefore, as discussed later, the role of copper(II) was further investigated.

3.5. Role of pH

The applied Landolt reaction is very sensitive to pH, t_L being inversely proportional to $[H^+]^2$ (see Eq. 3) [3]. An increase in the pH of the sample increases t_L and causes a positive error in the direct measurements. The studied tablets contain large amounts of carbonates or hydrogencarbonates and organic acids to induce a sparkling effect. These components also act as buffers. Depending on the actual composition, the pH of the sample solutions varied between 4.03 and 4.77. Because of the pH sensitivity, the direct method is expected to yield reproducible results only if the pH of the reaction mixtures is maintained at a well defined constant value. This laborious procedure can be avoided by using the standard addition technique. Since the added ascorbic acid does not alter the pH, in this case a constant pH value is maintained for each standard addition series by the well buffered sample.

3.6. Optimum conditions for analysis

According to preliminary experiments, the result of L-ascorbic acid determination was the most sensitive to the applied conditions in the case of Vitakid. Therefore, detailed studies were carried out with this product in order to optimize the analytical procedure. Measurements were carried out by using five different ascorbic acid-free base granules of Vitakid. The granules contained the following components: Granule I, vitamin mixture; Granule II, iron(II) compound and a portion of the organic acid; Granule III, hydrogencarbonate and dyestuff; Granule IV, metal salts and complexing agents; Granule V, the other portion of organic acid, sweetening agent, flavour and polyethylene glycol. Granules I–IV also contained D-mannitol.

From the granules equivalent amounts of a 3.5 g Vitakid tablet were weighed in and spiked with ca. 35 mg of solid ascorbic acid. The samples were dissolved in de-aerated distilled water. The solutions were purged with nitrogen immediately after dilution. Direct measurements and standard addition measurements, using 0.5 cm^3 of 5×10^{-3} ascorbic acid, were preformed. The results obtained for the five different granules are summarized in Table 3. As shown, in the direct measurements a significant positive error was obtained for Granule III and a smaller negative error for Granule IV. In agreement with the experimental results, the combintion of the

Table 4
Study of interferences with Granule IV

Interfering species	Added ascorbic acid (mg)	Direct measurement		Standard addition	
		Ascorbic acid (mg)	Recovery (%)	Ascorbic acid (mg)	Recovery (%)
Cu(II) ions	35.8	28.82	80.5	30.04	83.9
Mn(II) ions	35.1	37.39	106.5	36.48	103.9
D-Mannitol	35.1	34.33	103.7	33.99	102.4
Cu(II)+EDTA	35.3	35.61	100.9	36.08	102.2

listed errors gives an overall positive error for the analysis of Vitakid tablets.

In the case of Granule III, the error is due to a pH effect. Because of the large hydrogencarbonate ion content, stock solutions of Granule III have a large buffer capacity and relatively high pH (ca. 8.6). Standard ascorbic acid solution and stock solutions of other Granules are less buffered and their pH is in the acidic region, 2.50–3.82. As a result, the pH of the reaction mixture is 2.09 with Granule III and 1.85–1.92 with the other granules and standard ascorbic acid solution. On the basis of Eq. 3, the more than 100% error in the measured ascorbic acid concentration seems to be consistent with the noted ca 0.2 pH difference. The observed linear relationship between the determined ascorbic acid concentration and amount of Vitakid sample can be explained in a similar fashion. On increasing the amount of sample the hydrogencarbonate ion concentration and, as a consequence, the pH increased in the reaction mixtures. A higher pH means a longer Landolt time and a higher apparent ascorbic acid concentration.

The previous considerations are fully supported by the fact that the error of the direct measurements decreased to 4.1% when the pH of the Granule III stock solutions was adjusted to 3.3 with HCl solution prior to the analysis. The lack of significant deviation with the standard addition technique is also consistent with the above explanation.

In the case of Granule IV, both the direct and standard addition measurements gave erroneous results. In order to find the source of this error, the effects of the main components, i.e. Cu(II), Mn(II) and D-mannitol, were studied separately. In these experiments, the concentrations of the interfering species were the same as in the Vitakid tablets. The results in Table 4 clearly demonstrate that copper(II) is responsible for the significant negative error. As discussed earlier, the involvement of copper(II) in simple redox reactions with I^- and L-ascorbic acid would justify only about a 2.3% negative error. The observed more than fivefold larger error strongly suggest that Cu(II) may also act as a catalyst of the Landolt reaction. The study of the mechanism of this catalytic reaction falls outside the scope of the present work. However, it was shown that regardless of the method applied, i.e. direct or standard addition measurements, the effect of copper(II) can be completely avoided by adding EDTA solution in excess to the analysed samples.

The above results clearly demonstrate that the BrO_3^-–I^-–L-ascorbic acid Landolt reaction is suitable for the determination of vitamin C in the effervescent products provided that the experiments are carefully designed to eliminate the interfering effects. The reliability of the method was tested as follows. About 3.5 g of base Granule of Vitakid (an appropriate mixture of Granules I–V) and ca. 35 mg of crystalline ascorbic acid were weighed into a 100 cm^3 volumetric flask and 1 cm^3 of 0.1 mol dm^{-3} EDTA was added. The sample was dissolved in de-aerated water. A series of reaction mixtures were prepared as described in the Experimental section by adding 0, 0.2, 0.4, 0.6, 0.8 and 1.0 cm^3 of 5×10^{-3} mol dm^{-3} L-ascorbic acid stock solution. In these six-point standard addition measurements excellent linearity was observed

Table 5
Vitamin C content of different effervescent tablets

Tablets	Vitamin C content (mg/tablet)		RSD (%)	n
	Declared	Measured		
Béres Vitalin	60	63.08	2.0	7
Béres Vitakid	35	36.17	1.1	6
Plusssz Multivitamin	60	61.65	2.4	8
Plusssz C vitamin	75	82.09	2.8	7
Plusssz Vas+C	75	74.73	0.7	6
Ca-C 1000 Sandoz	1000	994.73	1.6	6

between t_L and the added ascorbic acid concentration. The determined vitamin C content of the samples varied between 100.73 and 101.65% of the added quantity. The RSD for seven samples was 0.7%.

The same procedure was used for the analysis of the effervescent tablets listed in Table 1. As shown in Table 5, the measured vitamin C contents are in excellent agreement with the declared values. It follows that the accuracy and reproducibility of the method are acceptable for most practical purposes. This simple chronometric method does not require expensive instrumentation. Therefore, it is recommended whenever the fast determination of vitamin C content is necessary at any stage of the manufacturing process, or to check the uniformity and stability of the end-products. The results may also serve as a basis to develop a flow injection analysis method for the in-process determination of L-ascorbic acid in a variety of pharmaceutical and food products (experiments are in progress).

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