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COMMUNICATION

Water Determination in Drugs Containing Ascorbic Acid

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ABSTRACT

A new rapid analytical method was applied for water determination in tablets of vitamin C, Ce De Calcium Veterinary, and C-Tamin-500 containing ascorbic acid and therefore is not amenable for direct Karl Fischer (KF) titration. The method is based on the consecutive titration first of ascorbic acid by a novel reagent and then of water by a conventional KF reagent (KFR) in the same sample and cell with the electrometric ''dead-stop'' location of the end point in both titrations. The new reagent consists of iodine, potassium iodide, and sodium acetate in nonaqueous medium. Estimated repeatability and accuracy of both water and ascorbic acid determination are satisfactory.

Key Words: *Analytical method validation; Ascorbic acid determination; Drugs in tablets; Karl Fischer titration; Water determination.*

INTRODUCTION

The pharmaceutical industry produces different drugs that contain synthetic and natural ascorbic acid (powders, tablets, capsules, suppositories, extracts, etc.). In the complex preparations and in many natural products, vitamin C is oxidized at exposure to air and light or at heating because of the ene-diol group presence in its structure (1). Vitamin C oxidation by iodine does not allow use of Karl Fischer (KF) titration for water determination in these preparations and products. Since the oxidation by air leads to water formation, such pharmacopoeial methods for water determination as azeotropic toluene distillation and ''loss on drying'' (2) are not relevant in these cases. At the same time, the well-known method for ascorbic acid determination is based on the oxidation of the ene-diol group by iodine (3). This method is better suited for the analysis of complex and painted samples

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since the USP method (2) does not require use of the indicator dye (2,6-dichloroindophenol) for the control of the redox reaction.

A novel reagent and rapid method were developed by us for the simultaneous determination of water and ascorbic acid in samples not amenable for direct KF titration (4–6). This method is based on the consecutive titration first of ascorbic acid by the novel reagent and then of water by a conventional KF reagent (KFR) in the same cell and sample with double burette and electrometric ''dead-stop'' location of the end point in both titrations. The method can be used for the analysis of drugs containing fillers or other matrix components (e.g., lactose, starch, talc, stearin, cellulose, etc.) that do not react with the novel reagent and KFR. Carbonates, bicarbonates, oxides of metals, and sulfites interfere. The novel method has the following advantages: rapidity, simplicity, and the use a single test portion for determination of two analytes without any blank and extra solvent. Evaluation of the validation parameters of the method was performed for three artificial samples of ascorbic acid and four kinds of purchased vitamin C tablets (7). The artificial samples of ascorbic acid were prepared by the intimate mixing of ascorbic acid with disodium tartrate dihydrate (to introduce the known quantity of water). The evaluated parameters of the method, such as accuracy, recovery, repeatability, reproducibility, and the limit of quantitation, satisfied requirements of the AOAC program (8). The method has been adopted as an AOAC Peer-Verified Method, number 1: 1998.

In the present paper, the novel method was applied for water and ascorbic acid determination in the tablets of C-Tamin-500 and Ce De Calcium Veterinary. The results of the application are compared with the data (7) previously obtained for the vitamin C tablets.

EXPERIMENTAL

Reagents and Materials

The Novel Reagent

The novel reagent consists of iodine, potassium iodide, and sodium acetate in a mixture of *N*,*N*-dimethylformamide (DMF) with methanol (1:3 v/v) (4). It is expedient to prepare and keep a concentrated reagent with titers of 0.02–0.05 mole iodine per liter, while it is better to use a diluted reagent, depending on the sample to be analyzed. Since the content of ascorbic acid in the C-Tamin-500 tablets is 13-fold more than in the Ce De Calcium tablets, it is worthwhile to use two diluted reagents (A and B). Reagent A consists of about 0.05 mole iodine, 0.075 mole potassium iodide, and 0.3 mole sodium acetate in a mixture of DMF with methanol (1:3 v/v) for analysis of the C-Tamin-500 tablets. Reagent B consists of about 0.004 mole iodine, 0.006 mole potassium iodide, and 0.3 mole sodium acetate in a mixture of DMF with methanol (1:3 v/v) for analysis of Ce De Calcium tablets. The reagents are ready to use 2 days after preparation, and then standardization of iodine and water contents is performed. The iodine titers of the reagents are determined against 0.1 N and 0.01 N sodium thiosulfate solutions daily for reagents A and B, respectively. The concentration of water traces (<0.15 mg H_2O/ml) is estimated weekly against anhydrous tin(II) chloride, about 30–50 mg or 3–5 mg for reagents A and B, respectively. The reagents are stored in airtight containers made of amber glass.

Karl Fischer Reagent

The KFR is prepared from Hydranal-Composite 1, which is diluted twofold with the mixture of dry methanol and Hydranal-Solvent in a ratio of 3:1 v/v. The titer of the diluted KFR is about 0.5 mg H_2O/ml. The titer of the KFR is established according to the known weight of water, Hydranal-Sodium tartrate-2-hydrate (15.66% $\pm$ 0.05% H_2O) or Hydranal-Standard 5.00 (5.00 $\pm$ 0.02 mg H_2O). Hydranal products are from Riedel-de-Haen, Seelze, Germany.

Analyzed Drugs

Three kinds of vitamin C tablets (Rekach Pharm. Ind. Ltd., Holon, Israel) were used: vitamin C-500, vitamin C-100, and vitamin C-50. Each tablet of C-500 weighs about 0.62 g and contains 500 mg (80%) of ascorbic acid; the rest is talc, starch, magnesium stearate, and stearin. Each tablet of vitamin C-100 and vitamin C-50 weighs about 0.4 or 0.12 g, respectively, and contains 100 mg (25%) or 50 mg (40%) of ascorbic acid, respectively. The rest is powdered cellulose, lactose, starch, calcium stearate, acacia, and talc as indifferent fillers.

Ce De Calcium Veterinary tablets (Taro Pharm. Ind. Ltd., Haifa, Israel). Each tablet weighs about 0.83 g and contains 50 mg (6.1%) of ascorbic acid, 500 IU of vitamin D, and 300 mg of calcium phosphate; the rest is cellulose, cocoa, and other indifferent fillers. These tablets have a light brown color.

The C-Tamin-500 tablets (Rekah Pharm. Ind. Ltd., Holon, Israel) each weigh about 0.63 g and contain 500

mg (79.4%) of ascorbic acid, starch, talc, magnesium stearate, stearic acid, and cellulose.

Apparatus

The titration system consists of a double burette with two 10-ml microburettes graduated in 0.02-ml divisions, a titration flask with two platinum electrodes, an electrometric ''dead-stop'' end-point detection system, and a means for protecting the reagent and the KFR against atmospheric humidity (4). A microweighing bottle of Teflon, 0.5 ÷ 1.5 ml, with a stopcock is used for weighing a test portion of water, Hydranal-sodium tartrate-2-hydrate, and a drug sample (20–120 mg). Gastight syringes (Hamilton Company, Reno, NV), 500 and 1000 ml, are used for taking the Hydranal-Standard 5.00 volume.

Sample Preparation

A tablet sample is gently powdered in a China mortar. During this operation, it is necessary to avoid high-speed mills since water can be lost. The sample, as a fine powder, is stored in a closed container at room temperature in a dark place.

Procedure

The dried titration flask is connected to a double burette, then the air is displaced from the flask by dry nitrogen application for 30 sec. The stopcock of the weighing bottle with the test portion is opened, and the sample is introduced via the flask side pipe (together with the bottle and its stopcock). Ascorbic acid is titrated completely against the novel reagent (first titration). The reagent volume spent for the titration is used for the calculation of the ascorbic acid content in the test portion. After the first titration, the total water content in the flask consists of the original amount of water in the test portion and that introduced during titration with the novel reagent. This total water content is determined against the KFR (second titration). At the beginning of the second titration, about 0.2–0.3 ml of the KFR are added to the flask for acceleration of the titration process. The KFR volume spent is used for the calculation of the water content in the analyzed sample. It is impossible to protect the titration flask completely from the atmospheric humidity; therefore, the end point of the titration is characterized by a stable indication of the voltage for 1–2 min with the stirrer on, which corresponds to the minimal iodine excess at the equivalent point.

Calculation

The ascorbic acid content is calculated as usually in titrimetry. The water content (C_w, %) is calculated from the formula

$$C_w = [V_{\mathrm{KFR}} - (F \times V_R)] \times T_{\mathrm{KFR}} \times 100/m \qquad (1)$$

where m is the mass of the test portion, V_{KFR} is the volume of the KFR spent for titration of the solution formed after the first titration (ml), V_R is the volume of the novel reagent spent for titration of the test portion (ml), F is the factor that corresponds to the volume of the KFR (ml) spent for titration of water traces in 1 ml of the novel reagent, and T_{KFR} is the titer of the KFR (mg H_2O/ml). The F value is calculated from two consecutive titrations of the same dry $SnCl_2$ sample by the novel reagent and then by KFR:

$$F = V^0_{\mathrm{KFR}}/V^0_R \qquad (2)$$

where V^0_{KFR} is the volume spent for the second $SnCl_2$ titration of the solution formed after the first titration (ml), and V^0_R is the volume of the novel reagent spent for the first titration (ml). The results of water and ascorbic acid determinations in the tablets of vitamin C, Ce De Calcium, and C-Tamin-500 are given in Tables 1 and 2.

RESULTS AND DISCUSSION

The application of the new method permits carrying out water determination by the KFR in a sample containing a strong interference: ascorbic acid. The first titration according to this method fulfills a double role: (*a*) determining the ascorbic acid concentration (an assay) and (*b*) preparing the working medium for the water determination by the KFR. It is known that the optimum rate constant of the KF reaction corresponds to pH values of the working medium over the range 5.5–8 (9). The concentration of sodium acetate for the preparation of the reagent is chosen so that its pH value responds to the pH of the KFR. Therefore, the transition from the first titration to the second one proceeds without a noticeable decrease of the KF reaction rate. However, the optimal concentration of sodium acetate in the reagent should be 0.2–0.3 mol/L (4) because otherwise the redox reaction between iodine and ascorbic acid will not proceed quickly enough. At a concentration of sodium acetate

Table 1

Results of Water and Ascorbic Acid Determination by the New Method

	X_{av} (%)	S_r (%rel)	S_{rc} (%rel)	B (%rel)	B_c (%rel)
Water					
Vitamin C-500	1.11	1.62	2.63	4.34	7.68
Vitamin C-100	4.94	1.23	2.10	3.55	9.17
Vitamin C-50	4.13	1.19	2.15	1.61	6.31
Ce De Calcium	3.32	2.44	2.23	0.91	6.52
C-Tamin-500	1.60	2.65	2.49	0.63	7.27
Ascorbic acid					
Vitamin C-500	79.76	0.40	1.38	−0.85	5.71
Vitamin C-100	25.84	1.01	1.64	2.23	4.79
Vitamin C-50	40.24	1.02	1.53	−1.34	4.48
Ce De Calcium	5.72	1.92	2.05	0.69	6.01
C-Tamin-500	79.53	0.63	1.38	−0.34	5.72

higher than 0.4–0.5 mol/L, a side reaction takes place, with slow consumption of the excess iodine even when ascorbic acid is titrated completely. This reaction is similar, probably, to the reaction between the acetoacetic ester disodium salt and iodine, which forms iodine acetoacetic ester as an intermediate compound (10).

For successful assay and water determination in tablets, the semimicroweight of the test portion is important, as well as the nonaqueous solvent, which ensures the quantitative extraction and/or dissolution of the analytes and reaction products. Polysaccharides, different kinds of cellulose, salts of stearic acid, stearin, starch, and talc usually are used as fillers for the preparation of the tablets. Since a majority of the fillers possesses relatively high heats of absorption (11), the complete dehydration and extraction time for assay and water determination depends on qualitative and quantitative composition of the tablets to be analyzed. For example, the titration time for ascorbic acid against the novel reagent is twofold to threefold less than that for water against the KFR, and the total titration time by both reagents is only 6–8 min for C-Tamin-50, while for Ce De Calcium, it is 18–20 min. Consequently, at high contents of the above-mentioned hydrated fillers in the tablets, the time necessary for complete extraction of the analytes from the matrix may be increased.

Table 2

''True'' Values of Water and Ascorbic Acid Content in the Drugs

	Water		Ascorbic Acid	
Drug	X_{tr} (%)	S (%rel)	X_{tr} (%)	S (%rel)
Vitamin C-500	1.06	2.82	80.45	0.30
Vitamin C-100	4.77	1.02	25.27	2.66
Vitamin C-50	4.06	1.33	40.78	1.12
Ce De Calcium	3.29	3.65	5.76	2.26
C-Tamin-500	1.61	4.06	79.80	0.14

The precision and accuracy of the water and ascorbic acid determination by the new method were evaluated from the data shown in Table 1.

As the ''true'' ascorbic acid contents in the vitamin C tablets, the results of the analyses (average of 10 replicates) by the USP method (2, p. 131) were used. Corresponding true values of the assay in the Ce De Calcium and C-Tamin-500 tablets were obtained (average of 5 replicates) by the classic iodometric titration (12). The true water contents for the tablets of vitamin C (average of 10 replicates), Ce De Calcium, and C-Tamin-500 (average of 5 replicates) were calculated as a difference between two titrations: the sum of the assay and water determination by the KF titration and the assay only. Such a technique for a calculation of the true water content is accepted since ascorbic acid is oxidized by iodine completely in aqueous medium, as well as in the nonaqueous one by the KFR (1). True values and corresponding relative standard deviations of replicates S are presented in Table 2.

Precision is evaluated on the repeatability level. Precision is characterized by the relative standard deviations of replicates S_r. The values of S_r for vitamin C tablets are calculated from the data obtained by 4 replicates per day for 5 days. All the data are ''homogeneous'' for different days and so were averaged (7). Therefore, for Ce

De Calcium Veterinary and C-Tamin-500 tablets, S_r values are calculated from the shortened experiment consisting of 5 replicates obtained during 1 day. From Table 1, one can see that, for water determination, $S_r < 3\%$rel, and for ascorbic acid determinations, $S_r < 2\%$rel for all vitamin C and other samples. These values satisfy the Horwitz criterion (8):

$$S_r < S_{rc} = 2^{(1-0.5 \log C)} \times 0.67 \quad (3)$$

where S_{rc} is the critical S_r value according to the Horwitz criterion, and C is the concentration of the analyte in decimal fractions.

Accuracy is characterized by the relative bias B of average results X_{av} obtained by the new method from the true values X_{tr}:

$$B = (X_{av} - X_{tr}) \times 100/X_{av} \quad \%\text{rel} \quad (4)$$

Its values satisfy the following criterion based on the normal distribution and the Horwitz one:

$$B < B_c\{P\} = U\{P\} \times S_{Rc} \quad (5)$$

where $U\{P\} = 1.96$ is the coefficient of the normal distribution at the level of confidence $P = .95$ and

$$S_{Rc} = 2^{(1-0.5 \log C)} \quad (6)$$

is the critical value of the standard deviation reproducibility S_R according to the Horwitz criterion.

So, the application of the method by these assessments is satisfactory also. Moreover, we are sure the new method can be applied for the analysis of any drug containing ascorbic acid that is free of components that react with iodine.

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