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Simultaneous kinetic spectrophotometric determination of citric and ascorbic acid by H-point standard addition method

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The H-point standard addition method was applied to kinetic data for simultaneous determination of citric and ascorbic acid or selective determination of ascorbic acid in presence of citric acid. The method is based on the difference in the rate of reaction of citric and ascorbic acid with copper(II)-ammonia complex. The linear dynamic ranges for the two analytes of citric and ascorbic acid are 0.80-1.15 × 10^2 and 0.70-10.00 mM, respectively. The proposed method was successfully applied for the determination of citric and ascorbic acid in some powdered drink mixtures and vitamin C tablet.

Keywords: Citric acid, Ascorbic acid, Copper(II)-ammonia complex, H-point standard addition method

IPC Code (s): G01J3/00, C07C59/265, C07D307/62

Citric acid is the most versatile and widely used organic acid in the field of foods and pharmaceuticals. It is often used as an antibacterial substance and as additive for the control of *p*H. It is used as an acidulate in beverages, confectioneries, in pharmaceutical syrups, elixirs, in effervescent powders and tablets, to adjust the *p*H of foods. It is a commonly used parameter in the analysis of food and pharmaceutical preparations. Analytical methods such as volumetric[1], enzymatic[2], spectrophotometry[3,4], high performance liquid chromatography (HPLC)[5-8] and gas-liquid chromatography[9] methods were used in determination of citric acid in different matrices.

Vitamin C or L-ascorbic acid, is an essential nutrient for health maintenance. Nearly all species of animals synthesize L-ascorbic acid and do not require it in their diets, but humans cannot synthesize the vitamin. For practical purposes, raw citrus fruits are good daily sources of L-ascorbic acid. It is an antimicrobial and antioxidant in foodstuffs. Therefore, food products are well accepted by the consumer when a high content of vitamin C is indicated. Titrimetry[10], HPLC[11], spectrophotometry[12,13], kinetic[14], voltammetry[15] and flow injection[16] methods have been used in the determination of ascorbic acid.

Spectrophotometric determination of citric acid[17] and ascorbic acid[18] on the basis of reaction with Cu^{2+}-NH_3 complex, have been also made. In spite of many papers published on the determination of citric and ascorbic acid, individually, only a few paper about simultaneous determination of citric and ascorbic acid[19-22] have been published. One kinetic spectrophotometric method employs the Kalman filter[19] while some others are chromatographic methods[20-22].

The H-point standard addition method (HPSAM) is a modification of the standard addition method that transforms the incorrigible error resulting from the presence of a direct interference in the determination of an analyte into a constant systematic error. This error can then be evaluated and eliminated. This method also permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. The basis of the method was estabilished previously[23,24]. Absorbance increments as analytical signals were used when only the analyte concentration was required[25].

Two variants of HPSAM can be used for the treatment of kinetic data[26]. One is applied when the reaction of one component is faster than that of the other or the latter dose not take place at all. This variant of the method is based on the assumption that only analyte X evolves with time and the other species Y or interferents do not affect the analytical signal with time. In this case the variables to be fixed are two times t_1 and t_2 at which the species Y, which

does not evolve with time or over the range between these times, should have the same absorbance. The other variant of the method is used when the rate constants of the two components are time-dependent. In this case, the two species in a mixture, X and Y, both evolve with time.

In this study the first variant of kinetic based HPSAM is suggested as a simple, precise and accurate method for simultaneous determination of citric and ascorbic acid, based on the different reaction rate with Cu^{2+}-NH_3 complex.

Experimental Procedure

Apparatus

UV-Visible absorbance digitized spectra were recorded on a UV-VIS GBC 916 spectrophotometer. Measurements of *p*H were made with a PMT 1003 *p*H-meter using a combined glass electrode. The computations were made on a Pentium 133 MHz computer.

Reagents

All solutions were prepared with double distilled water. Chemicals used were of analytical grade and were purchased from E. Merck.

Cu(II)-NH_3 complex solution was prepared by dissolving 3.1210 g $CuSO_4.5H_2O$, 2.8590 g ammonium chloride and 16.0 mL concentrated ammonia in 50 mL volumetric flask.

Stock citric acid (0.5 M) and ascorbic acid (0.1 M) were prepared by dissolving appropriate amounts of citric acid and ascorbic acid in water. Working standard solutions were prepared by dilution of these solutions.

Method

One mL of Cu(II)-NH_3 complex solution and appropriate volumes of the stock citric and ascorbic acid solutions were transferred in to flask, and diluted to 10.0 mL with water. The blank reagent was prepared by dilution of 1.0 mL to 10.0 mL with water. The absorbance measurements were made at 600 nm with 1s time intervals for each sample after preparation of a sample solution against of blank sample. Simultaneous determination of citric and ascorbic acid with HPSAM was performed by measuring absorbances at just 30 and 180 s after initiation of reaction for each sample solution. The concentration ranges of citric and ascorbic for construction of HPSAM calibration graph were 0.800-1.15×10^2 and 0.70-10.00 mM, respectively.

Results and Discussion

Cu(II)-NH_3 complex is a coloured complex of copper(II). Also, Cu(II)-citrate complex is another complex for which λ_{max} is greater than Cu(II)-NH_3 complex. On the other hand, stability of the citrate complex of copper(II) is greater than Cu(II)-NH_3 complex.

$$Cu^{2+} + 4NH_3 \leftrightarrow Cu(NH_3)_4^{2+} \quad k_{1,2,3,4} = 9.33 \times 10^{-13} \qquad \ldots (1)$$

$$Cu^{2+} + Cit^{3-} \leftrightarrow CuCit^{-} \quad k = 6.17 \times 10^{-15} \qquad \ldots (2)$$

(k is dissociation constant)

The absorbance spectrum of Cu(II)-NH_3 complex is shown in Fig. 1a, λ_{max} of this complex is about 600 nm. By addition of citric acid to the solution of Cu(II)-NH_3 complex, it is observed that the absorbance at λ_{max} of Cu(II)-NH_3 complex (600 nm) is decreased (due to decomposition of this complex) and simultaneously the absorbance at λ_{max} of Cu(II)-citrate complex (740 nm) to be increased by the formation of this complex (Fig. 1b).

Also the absorbance at 600 nm was decreased by addition of ascorbic acid, due to reduction of Cu(II)-NH_3 complex (Fig. 1c). On the other hand, Cu(I)-NH_3 complex is an unstable complex and oxides to the Cu(II)-NH_3 complex when it is in contact with O_2 of air, as by shaking of the Cu(I)-NH_3 complex solution in the presence of air, its absorption spectrum was changed and conformed to the Cu(II)-NH_3 spectrum[18]. But O_2 causes no serious interference, provided the measurement is carried out

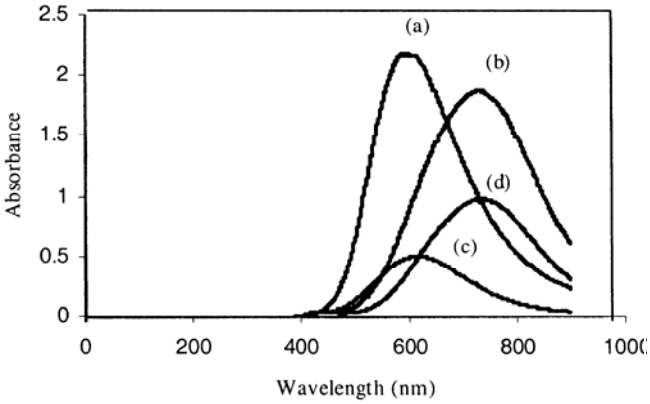

Fig. 1—The absorption spectra of (a) 0.025 M Cu^{+2}-NH_3 complex; (b) a plus 50.0 mM citrate; (c) a plus 10.0 mM ascorbic acid after 3 min; (d) a plus 50.0 mM citric acid and 10.0 mM ascorbic acid after 3 min.

immediately and without shaking[18]. Also, the measurements were made in closed-door cells.

It should be mentioned that using Cu(II)-NH_3 complex instead of Cu(II) decreases interferences from other species such as OH^- or other precipitants or complexing agents.

In the preliminary investigations it was observed that there is a difference between the rate of reactions of citric and ascorbic acid. In fact two processes are involved; the fast complex formation between citric acid with Cu(II)-NH_3 complex and relatively slow reduction of Cu(II)-NH_3 complex. So, the kinetic based HPSAM is a good selection for simultaneous determination of citric and ascorbic acid.

Effect of variables

In order to find the optimum concentrations, the effect of Cu(II) and ammonia/ammonium buffer concentration were studied. For the selection of the optimum concentration of this complex, the concentration of Cu(II) and ammonia and ammonia to ammonium ratio were varied in different solutions with citric acid or ascorbic acid and the decrease in absorbance in 600 nm was measured for citric acid (the absorbance is constant in different times), but for the ascorbic acid the change in the absorbance in the fixed time period (30-180 s) was measured. The study on the influence of Cu(II) concentration on the rate of ascorbic acid reaction showed that the maximum change in absorbance in the fixed time period occurred at 0.025 M of Cu(II), therefore amount of 0.025 M was selected for Cu(II) concentration, because it has relatively good sensitivity for both citric and ascorbic acid.

The ammonia/ammonium buffer was used not only for the *p*H adjustment but also as a complexing agent. In order to determine its optimum concentration, various concentration of ammonia (0.2-1.4 M) at constant ammonia to ammonium ratio (1:1) were added to the Cu(II) (0.025 M) solution. The results show 0.43 M ammonia has relatively good sensitivity for both citric and ascorbic acid, and therefore this concentration was used in the further studies as an optimum concentration for ammonia.

For adjustment of *p*H at optimum amount, a series of the solutions containing 0.025 M Cu(II) and 0.43 M ammonia was selected and the ammonia to ammonium ratio was varied. It is evident that at *p*H<8.5, the formation of the ammonia complexes are not quantitatively possible and at *p*H>11.0 the oxidation of the ascorbic acid by O_2 is rapid[18]. From these results a 4:1 ratio (ammonia to ammonium) was selected as optimum ratio, whose sensitivity is suitable for both the acids and the buffer capacity is good.

Applying of HPSAM

In order to test applicability of HPSAM to the simultaneous determination of citric and ascorbic acid the kinetic curves for each of them and for their mixture were drawn (Fig. 2). As shown when ascorbic acid is selected as the analyte, it is possible to select several pairs of times where there are the same absorbance values for citric acid. The pair of time, which gave the highest accuracy, was selected for further work. The criteria for choosing the optimum time pair were as follows. The best pair of time should give the greatest slope increment[27] and also the change in absorbance related to citric acid concentration is negligible. For this reason the time pair 30-180 s was employed for obtaining the highest accuracy. Figure 3 gives a sample of H-point standard addition calibration curve constructed at two selected times.

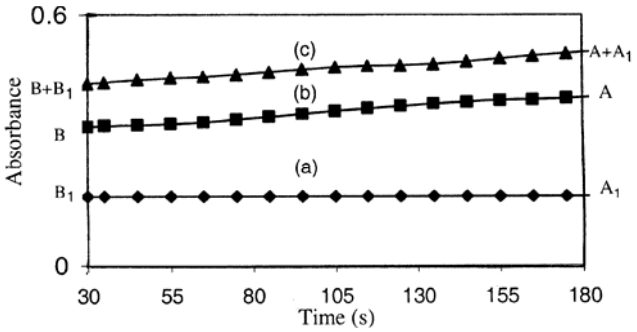

Fig. 2—Kinetic curve for (a) 20 mM citric acid; (b) 4 mM ascorbic acid; (c) mixture of 20 mM citric acid and 4 mM ascorbic acid.B_1 and A_1 are the absorbances of citric acid in two selected times 30 and 180 s and B and A are absorbances corresponding to ascorbic acid at 30 and 180 s, respectively.

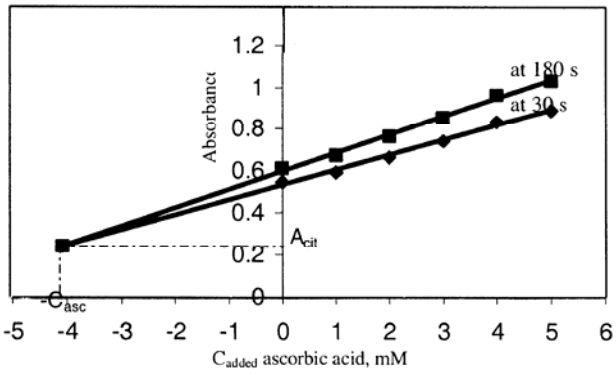

Fig. 3—Plot of HPSAM for simultaneous determination of ascorbic acid (4 mM) and citric acid (20 mM).

According to the theory of HPSAM at H-point[26], C_H (concentration of ascorbic acid at H-point) is independent of the concentration of citric acid and so, A_H (absorbance of citric acid at H-point) is also independent of the concentration of ascorbic acid. Figures 4 and 5 clearly show the effect of change in concentration of citric and ascorbic acid on the position of H-point. So, again as shown in Fig. 4, the value of A_H is independent of the amounts of ascorbic acid in the sample. This analytical signal enables calculation of the concentration of citric acid from a calibration curve.

An absorbance increment as an analytical signal can be employed in another version of HPSAM to allow the ascorbic acid concentration to be calculated with no systematic, constant or proportional error. The plot of $\Delta A_{t1\text{-}t2}$ -C $_{added}$ against the added analyte concentration will have a constant point ($-C_H$, 0)[26]. Thus, for the determination of ascorbic acid in presence of citric acid, the absorbance increment as an analytical signal was used. The application of the HPSAM in the $\Delta A_{t1\text{-}t2}$ -C $_{added}$ variant yields the concentration of ascorbic acid directly from the intercept on the x-axis. However, in order to ensure the absence of constant and proportional errors from the calculated concentration, all the possible ΔA_{t1-t2} - C_{added} lines for ascorbic acid should intersect at the same point, namely, that corresponding to the unknown concentration, C_H, as this would indicate that the time evaluation of the matrix would be a horizontal line. Figure 6 and Table 1 show the results obtained by employing this version of HPSAM on the synthetic mixtures of citric and ascorbic acid. The results obtained by this procedure were in good agreement with those given above.

Accuracy, precision and selectivity

Under the optimum conditions, simultaneous determination of different binary mixtures of citric and ascorbic acid were made using HPSAM. As Table 2 shows, the accuracy of the results is satisfactory. To check the reproducibility of the method, five replicate experiments were made and the relative standard deviation (RSD) was obtained for the mixtures. The results are given in Table 3. The precision of the results is satisfactory.

The effects of some organic acids, some of cations, anions, sugars and preservatives such as benzoic acid on the determination of 10.00 mM of citric acid and 2.00 mM of ascorbic acid were studied with the optimized conditions described above. The tolerance

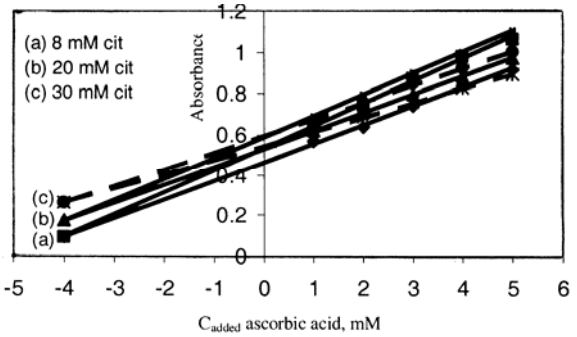

Fig. 4—Plots of HPSAM for fixed ascorbic acid concentration (4 mM) and different concentration of citric acid.

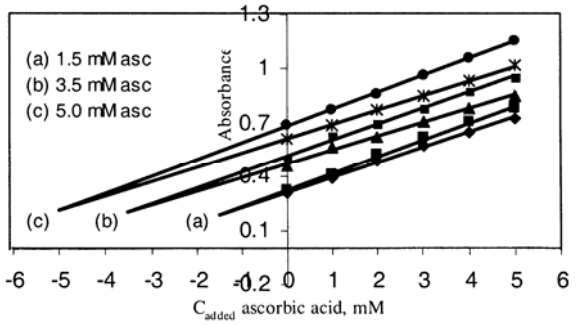

Fig. 5—Plots of HPSAM for fixed citric acid concentration (25 mM) and different concentration of ascorbic acid.

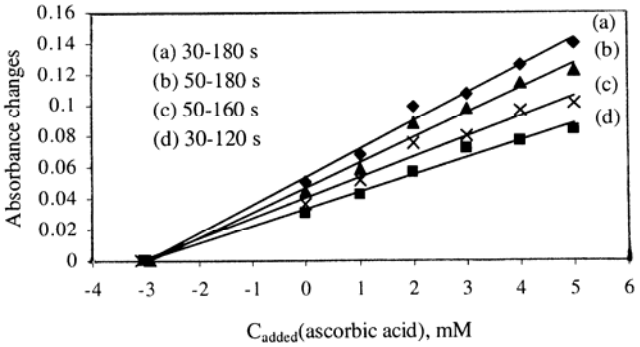

Fig. 6—ΔA versus added ascorbic acid concentration at different time intervals at 600 nm for synthetic mixtures containing 20 mM citric acid and 3 mM

Table 1—Application of signal increment version of HPSAM in a synthetic mixture

	Time interval (s)			
	30-180	50-180	50-160	30-120
Found ascorbic acid concentration (mM)	3.01	2.93	3.11	3.04

Actual concentration of ascorbic and citric acid were 3 and 20 mM.

Table 2—Results of five experiments for the analysis of ascorbic and citric acid

A-C equation	R	Present in sample (mM)		Found (mM)	
		Ascorbic acid	Citric acid	Ascorbic acid	Citric acid
$A_{180} = 0.1050C_I + 1.0875$ $A_{30} = 0.0901C_I + 0.9750$	0.9991 0.9987	7.00	35	7.50	33
$A_{180} = 0.0873C_I + 0.6107$ $A_{30} = 0.0758C_I + 0.5527$	0.9997 0.9996	5.00	20	5.04	20
$A_{180} = 0.1033C_I + 0.416$ $A_{30} = 0.088C_I + 0.3692$	0.9984 0.9985	3.00	10	3.06	10
$A_{180} = 0.1033C_I + 0.5894$ $A_{30} = 0.0887C_I + 0.5304$	1 1	4.00	20	4.04	20
$A_{180} = 0.1019C_I + 0.4928$ $A_{30} = 0.0887C_I + 0.4546$	0.9999 0.9993	3.00	25	2.89	24

Table 3—Results of five replicate experiments for the analysis of ascorbic and citric acid

A-C equation	R	Present in sample (mM)		Found (mM)	
		Ascorbic acid	Citric acid	Ascorbic acid	Citric acid
$A_{180} = 0.0953C_I + 0.5905$ $A_{30} = 0.0799C_I + 0.5287$	0.9984 0.9977	4	25	4.01	25
$A_{180} = 0.0967C_I + 0.6139$ $A_{30} = 0.0793C_I + 0.5456$	0.9995 0.9973	4	25	3.93	28
$A_{180} = 0.0981C_I + 0.5999$ $A_{30} = 0.0821C_I + 0.5357$	0.9982 0.9992	4	25	4.01	25
$A_{180} = 0.105C_I + 0.5891$ $A_{30} = 0.0924C_I + 0.5403$	0.9959 0.9942	4	25	3.87	22
$A_{180} = 0.0893C_I + 0.5506$ $A_{30} = 0.0763C_I + 0.4995$	0.9985 0.9974	4	25	3.93	24
Mean				3.95	24.8
Standard deviation				0.06	2.17
RSD(%)				1.54	8.75

limit is defined as the of foreign-ion concentration causing an error smaller than 5.0% for the simultaneous determining these acids. The results are presented in Table 4. The results indicate that the presented method is very selective.

Application

1.25 g of lemon and orange powders and one tablet of vitamin C were transferred into a 250 mL volumetric flask and dissolved in water, then made up to the mark with distilled water. Five milliliters of this solution were analyzed by HPSAM. The method, which has been proposed by Samuella and Dale[10], was used as standard method for determination of citric acid and ascorbic acid in the samples. The obtained concentrations for citric and ascorbic acid have a good agreement with standard method (Table 5). The good agreement between these results indicate the successful applicability of the proposed

Table 4—Tolerable concentration of the various substances in simultaneous determination of citric and ascorbic acid by the proposed method

Substance	Tolerable concentration (mgL^{-1})
Glucose	30000
Oxalate	2500
Maleate	4000
Sulphosalicylate	25000
Benzoate	35000
Tarterate	4000
Borate	28000
Magnesium	2500
Zinc	1000
Potassium	50000
Nickel	1000
Iron(III)	50
Calcium	1000
Nitrate	50000
Sulphate	50000
Disodium hydrogen phosphate	60000*

*Maximum concentration tested

Table 5—Determination of ascorbic and citric acid in several real sample solution

Com-pound	Citric acid (mM)		Ascorbic acid (mM)	
	By this method	By standard method[10]	By this method	By standard method[10]
Lemon powder	15.6 ± 0.9	15.6 ± 0.2	4.9 ± 0.1	5.1 ± 0.2
Orange powder	14.4 ± 0.8	14.8 ± 0.1	1.9 ± 0.1	2.2 ± 0.3
Vitamin C tablet	2.5 ± 0.8	2.4 ± 0.1	9.9 ± 0.8	9.5 ± 0.3

models for simultaneous determination of citric and ascorbic acid in the real samples.

Conclusion

In this study, HPSAM method for the simultaneous determination of citric and ascorbic acid based on decomposition of the Cu(II)-NH_3 complex has been presented. This method is fast, inexpensive and reproducible. The proposed method was used for the determination of citric and ascorbic acid in drink powder mixtures and vitamin C tablet and reliable results were obtained. High selectivity of the method allows it to be applicable in different samples and no serious interference was found.

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