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AUTOMATIC SPECTROPHOTOMETRIC PROCEDURE FOR DETERMINATION OF L-ASCORBIC ACID BASED ON REDUCTION OF IRON(III)-THIOCYANATE COMPLEX

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Abstract

A very simple spectrophotometric method has been developed for indirect determination of L-ascorbic acid using flow injection system based on the redox reaction between iron(III)-thiocyanate complex and L-ascorbic acid in acidic medium. A negative peak results from an injection of L-ascorbic acid into an iron(III)-thiocyanate complex carrier stream when absorbance is monitored at 462 nm. The height of the negative peak is proportional to the concentration of L-ascorbic acid in the sample. Figures of merit such as a relative standard deviation of 2.0% (n=6), linearity range up to 100 μg/mL and detection limit of 0.36 μg/mL were obtained. No significant differences at the 95% confidence level were observed in comparison with results obtained by a manual procedure. The proposed system allowed the determination of L-ascorbic acid in pharmaceutical formulations and foods.

Key words: Indirect determination, L-ascorbic acid, Iron(III)-thiocyanate, Flow injection analysis, spectrophotometric method

Introduction

L-Ascorbic acid (AsA) is an essential nutritious substance for human body participating in many different biological processes. It is found extensively in various vegetables and fruits and is used clinically in the treatment and prevention of scurvy, drug poisoning, liver disease, allergic reaction and atherosclerosis. A fast, selective and automated method for AsA determination is of importance in routine analysis. Various methods and techniques have been employed for its measurement, such as potentiometeric titration,[1] various modified electrodes,[2-5] sol gel,[6] high-performance liquid chromatography[7,8] and a combination of various other reagents. But some of them have low sensitivity; some of them need rigorous conditions, are expensive and time consuming. Therefore, an accurate, rapid and simple method for determination of AsA is desirable.

Furthermore, the use of flow injection (FI) makes any analytical method more attractive. Flow injection requires very little sample handling or manipulation and this eliminates many of the stringent clean procedures often necessary for standard AsA determinations. FI constitutes the most advanced form of solution manipulation available to analytical chemists for mixing and transporting the reagents and products of a chemical reaction to the point of measurement. A large number of flow injection techniques with different detection systems have been published for L-ascorbic acid measurement such as background correction with solid-phase iodine,[9] flame atomic absorption spectrometry,[10-12] spectrophotometry,[13,14] automated potentiometry and spectophotometric titration.[15,16]

In recent years, combination of iron-complexes system has been utilized for the indirect spectrophotometric determination of several pharmaceuticals.[17–21] To the best of our knowledge, however, there is no work in the literature reported about the application of iron(III)–thiocyanate system for the determination of pharmaceuticals.

The present work aims to demonstrate a simple, fast, accurate, precise, sensitive and inexpensive method suitable and convenient for the determination of ascorbic acid in a flow injection system using the estimation of the unreacted amount of iron(III)-thiocyanate from a known excess of iron(III)-thiocyanate as an oxidant for L-ascorbic acid. It has been applied for the determination of ascorbic acid in pharmaceutical formulations and foods.

Experimental

Reagents and samples

All chemicals were of analytical grade. Freshly distilled and deionized water was used throughout. All the reagents were purchased from Merck (Darmstatt, Germany). The sample and reference solutions were prepared daily and stored in amber bottles to avoid oxidation by light. An iron(III)-thiocyanate complex stock solution containing 0.01 mol/L $Fe(SCN)_6^{3-}$ [22] was prepared by adding excess KSCN- 5.060 g (0.2 mol/L), to 0.683 g (0.01 mol/L), $FeCl_3 \cdot 6H_2O$, with $[SCN^-]/[Fe(III)] = 20$ in 100 mL distilled water and diluting to 250 mL by nitric acid (0.2 mol/L). A 2×10^{-4} mol/L iron(III)-thiocyanate complex used as carrier was prepared by dilution of stock solution. L-Ascorbic acid stock solution 1000 μg/mL (5.73×10^{-3} mol/L) was prepared freshly before measurement

by dissolving 0.1000 g of ascorbic acid in 100 mL of water. 2,6-Dichlorophenolindophenol (DCPIP) solution (1×10^{-3} mol/L) was prepared by dissolving 0.0300 g of reagent in 2.5×10^{-3} mol/L $NaHCO_3$ and diluting to 100 mL in a calibrated flask.

Apparatus

A schematic diagram of the single-line flow system used in this work is given in (Fig.1). A six-way injector valve with a 110 μL loop, IV, was used. The carrier delivery and the flow rates were adjusted with a variable-speed pump, P, (LC22 pump, Bruker, Germany). Manifold lines consisted of 0.8 mm i.d. polyethylene tubing. The injection valve was kept in the load position for the first 10s of every run to load the sample loop, after which it was switched to the inject position to place the sample plug into carrier stream. The valve was kept in the inject position for further 30 s to ensure that the entire sample was flushed out of the sample loop. This was followed by switching the valve into the load position to fill the sample loop for the next run. After being placed in the carrier stream, the sample zone was pumped through the reactor, R, (80 cm polyethylene tubing with 0.8 mm i.d.). AsA in the sample zone reacted with iron(III)-thiocyanate complex in the reactor and the reaction was monitored at 462 nm with a variable wavelength monitor, D, (UV-detector, Knauer, Germany) equipped with a flow cell with 10 mm optical path-length. The whole procedure, from sample pumping to detection system, data processing and storage was computer-controlled via the chromstar software (Bruker, Germany) except the injection valve, which had to be operated manually.

Procedure

Conditioning of the flow injection system involved pumping 0.2 mol/L HNO_3 through the reactor for auto zero of the detector (Fig. 2a) and was followed by pumping of carrier stream solution (2×10^{-4} mol/L iron(III)-thiocyanate complex in 0.2 mol/L HNO_3) and waiting for baseline of detection system at 462 nm to reach a steady state (Fig. 2b). After conditioning the sample solutions, (AsA), were injected into the carrier stream**.** The concentration of AsA, in samples is proportional to the negative peak height. The computer read the generated negative peak signals coming from the detector. When L-ascorbic acid solution was inserted into the carrier stream, the magnitude of the

signal, negative peak, increased as a result of the reaction of L-ascorbic acid with iron(III)-thiocyanate complex. The signal acquires a negative polarity in an excess of the iron(III)-thiocyanate complex solution.

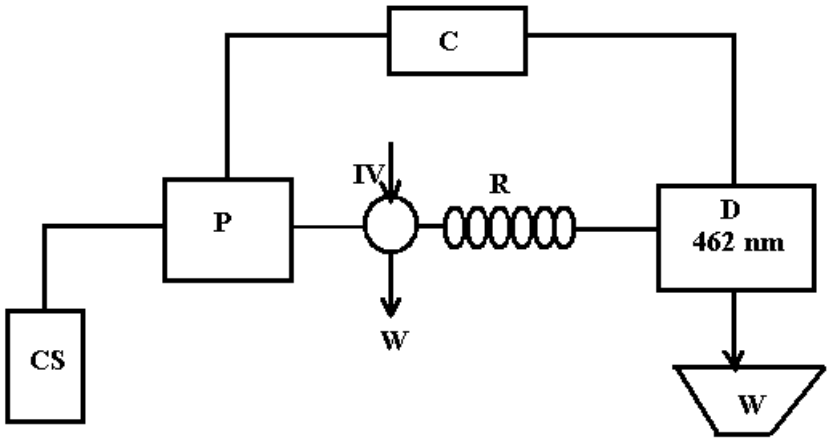

Figure 1. Schematic diagram of the flow-injection system. CS: carrier stream, P: pump, IV: injection valve, R: reactor, D: detector, C: computer, W: waste.

Vitamin C tablets

Ten tablets of vitamin C drug were accurately weighed, ground, powdered and dissolved in doubly distilled water. The content of the flask was shaken for 15 min and then residual solid was filtered and washed with water.[23] Titration with DCPIP was employed to determine the concentration of AsA in the tablets for validation purposes.[24] The samples were diluted appropriately to obtain concentrations within the working range of the method and assayed.

Real samples, fruit juices and foods

Fresh fruit juices, fruit juice made from concentrate, and flu remedies vitamin C sugar were selected. The foods such as orange and lemon were squeezedand then they were filtered through a dry Whatman No 1. filter. The juice obtained was diluted quantitatively with 0.2 mol/L HNO_3 for analysis. For the analysis of vegetables, samples of cucumber (30 g) and tomato (20 g) were cut into small pieces and pounded into a paste with 20 mL of 0.2 mol/L HNO_3 in a mortar box with a pestle, and then diluted to 100 mL with water. The supernatant was filtered through a dry Whatman No 1 filter.

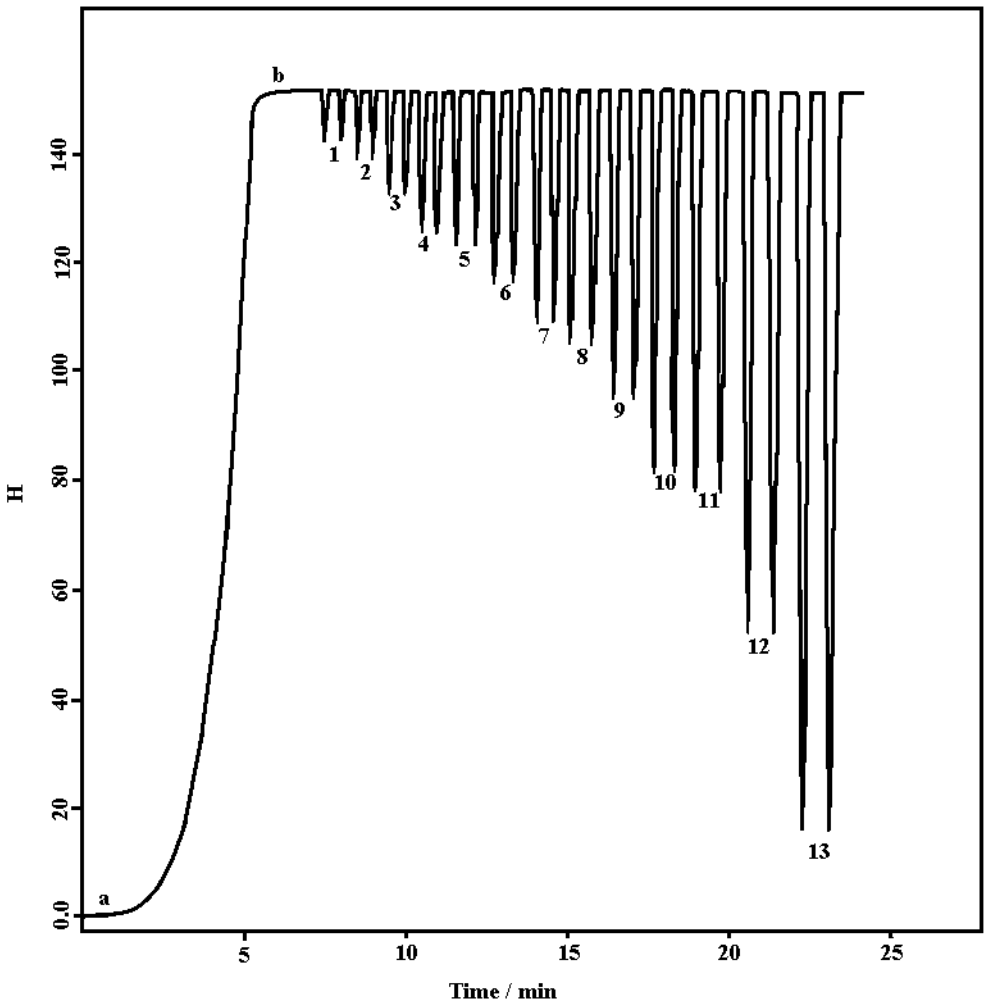

Figure 2: Recorded signals for standard solutions of L-ascorbic acid, 1) 3, 2) 7, 3) 12, 4) 17, 5) 19, 6) 25, 7) 30, 8) 33, 9) 40, 10) 50, 11) 54, 12) 75, 13) 100 μg/mL.

Results and Discussion

Oxidation Of L-ascorbic acid with iron(III)-thiocyanate

In this work, we used iron(III)-thiocyanate system for the determination of ascorbic acid in a flow injection system. The oxidation of L-ascorbic acid with a known excess of iron(III)-thiocyanate in nitric acid medium (0.2 mol/L) is indicated by the following chemical equation:

$$Fe(SCN)_6^{3-}\ (\text{excess}) + C_6H_8O_6 \longrightarrow Fe^{2+} + SCN^- + C_6H_6O_6 + 2H^+ + Fe(SCN)_6^{3-}$$

(Unreacted, λ_{max}= 462 nm)

Unreacted iron(III)-thiocyanate complex is detected spectrophotometrically at 462 nm as a step for the indirect determination of ascorbic acid. The concentrations of iron(II), SCN^- and dehydroascorbic acid ($C_6H_6O_6$) produced in the reaction are so small, that do not interfere in the measurement at 462 nm. L-Ascorbic acid, when added in increasing amounts, consumed iron(III)-thiocyanate and decreased the iron(III)-thiocyanate complex absorbance. Consequently, there is a concomitant fall in the iron(III)-thiocyanate concentration. In the flow injection system with carrier stream of iron(III)-thiocyanate complex, the negative peak height is found to increase linearly with increasing concentration of ascorbic acid, which forms the basis for its determination.

Selection of acid concentration and acid type

It is known that iron(III)-thiocyanate complex is stable in acidic media.[25] The optimum acidity of the solution with HCl, H_2SO_4, $HClO_4$ or HNO_3 lies within the concentration range 0.05-1 mol/L.[26] The following media have been tried in the proposed experiments: sulfuric acid, hydrochloric acid, perchlorate acid and nitric acid solutions. Analytical parameters of these acids have been studied and reported in Table 1. It was found that the sensitivity of reaction is very low in HCl and H_2SO_4 solutions; and decreasing of absorbance of iron(III)-thiocyanate complex is strong in HNO_3 and $HClO_4$ solutions. But the sensitivity is higher and the reproducibility is better only in nitric acid. Therefore, nitric acid was selected as the best reaction medium.

The negative peak height increased with increasing of acid concentration in the range of 0.05-0.2 mol/L and then, it was constant up to 1 mol/L. A nitric acid concentration of 0.2 mol/L was chosen for better sensitivity of experiments.

Table 1. Analytical parameters of the proposed method for different mineral acids.

Acid[a]	Intercept (a)[b]	Slope (b)[b]	correlation coefficient (r)	detection limit (μg/mL)
H_2SO_4	2.89	0.47	0.9922	0.81
HCl	2.94	1.02	0.9943	0.50
$HClO_4$	3.54	1.08	0.9955	0.39
HNO_3	4.04	1.36	0.9998	0.36

[a] Acids concentration and initial iron(III)-thiocyanate complex concentration were 0.2 mol/L and 2×10^{-4} mol/L, respectively. [b] The slope is obtained from equation; $H = a + b \times C_{AsA}$, where H is the negative peak height for concentration of L-ascorbic acid (C_{AsA}), a is intercept and b is slope.

Flow rate

The contact time between the sample zone containing AsA and the carrier solution (2×10^{-4} mol/L iron(III)-thiocyanate complex in 0.2 mol/L HNO_3) inside the reactor is very important for the reaction to proceed sufficiently close to the completeness. As this depends on the flow rate of the sample zone through the reactor, a study of the flow rate of the carrier stream was conducted. Flow rates between 0.25 and 1.75 mL/min were evaluated. This optimization is shown in Fig. 3. The highest analytical signal (negative peak height) of the signal increases from a flow-rate of 0.20 to a flow rate of 0.75 mL/min, decreasing slowly afterwards. A carrier flow rate of 0.75 mL/min was selected in order to obtain maximum sensitivity and minimum residence times. Under these conditions, at least 90 injections per hour can be performed.

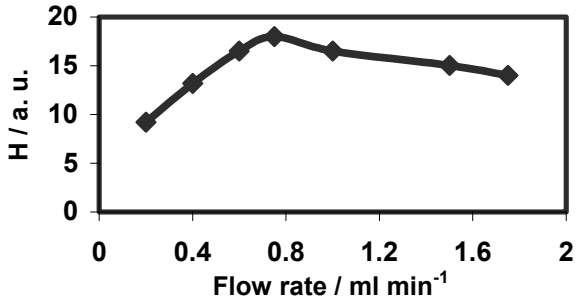

Figure 3. Influence of the carrier flow rate on analytical signal, Carrier = 2×10^{-4} mol/L iron(III)-thiocyanate complex in 0.2 mol/L HNO_3, reactor length = 80 cm and [AsA] = 10 μg/mL.

Reactor length

Finally the negative peak height of the analytical signal at a fixed AsA concentration, iron(III)-thiocyanate complex in 0.2 mol/L HNO_3 as carrier stream and flow rate is dependent upon the length of the reactor. The response of the system was studied by varying the reactor length between 30 to 100 cm with the i.d. fixed at 0.8 mm. The results are shown in Figure 4. The negative peak height significantly increases from a length of 30 cm to a length of 80 cm, remaining nearly constant afterwards. It seems that the reactor length of 80 cm gave sufficient time to the amount of injected AsA to make a complete reaction with iron(III)-thiocyanate complex. Therefore, 80 cm was chosen as optimum reactor length.

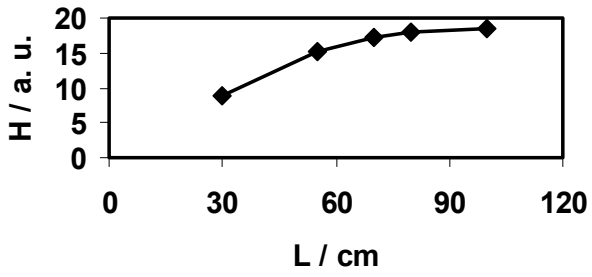

Figure 4. Influence of the reactor length on the analytical signal. Carrier = 2×10^{-4} mol/L iron(III)-thiocyanate complex in 0.2 mol/L HNO_3, Flow rate= 0.75 mL/min and [AsA]= 10 μg/mL.

Evaluation of the method

Using the experimental conditions described above, the calibration graph is linear up to 100 μg/mL and is described by the equation: $H= 4.04 + 1.36C_{AsA}$, r = 0.9998, n = 13 where H is the negative peak height in arbitrary units, C_{AsA} is the analyte concentration (μg/mL) r is the correlation coefficient and n represents the number of determinations. The detection limit (DL) was calculated using the equation $DL=3S_{bk}/m$, where S_{bk} is the standard deviation of the blank and m is the slope of the calibration graph. The calculated DL was found to be 0.36 μg/mL. The precision and accuracy of ten replicate analyses of a series containing various amounts of ascorbic by the variable-time method is shown in Table 2.

Table 2. Precision and accuracy of the method.

[AsA] present (μg/mL)	[AsA] found (μg/mL)	RSD % (n=8)	Relative error %
3.0	2.99	1.99	-0.33
9.0	9.1	1.36	1.112
15.0	15.1	1.44	0.67

Recovery tests

Recovery tests using the proposed method were performed using three different samples, and the test for each sample was carried out in triplicate. As shown in Table 3, the recoveries of ascorbic acid added to Cucumber, tomato juice, orange juice and lemon juice were all close to 100%. The results of the recovery tests are very good.

Table 3. Results of recovery test.

Sample	[AsA] added	[AsA] found[a]	Recovery
Cucumber	0.0	2.68	–
	10	13.0	103.2
Tomato juice	0.0	15.4	–
	10.0	25.1	97.0
Orange juice	0.0	57.8	–
	10.0	68.0	102.
Lemon juice	0.0	50.1	–
	10.0	60.2	101.0

[a] mg/100 g and average of five determinations.

Interference study

A study of interference for AsA determination was performed with samples containing 10 μg/mL L-ascorbic acid and an acceptable relative deviation of ΔH set to less than ± 5%. No interference is observed from organic compounds such as citric acid, malic acid, lactic acid, tartaric acid, fumaric acid, sorbic acid, glucose, fructose and saccharose (Table 4).

Table 4. Tolerance towards foreign compounds.

Additive type	Tolerance[a]
Citric acid, Urea	400
Mannitol	350
Glucose, Succinic acid, Malic acid, Sorbic acid, Fumaric acid, Calcium chloride, Sodium chloride, Sucrose, Fructose, Lactose, sodium acetate	260[b]
Maleic acid, Benzoic acid Salicylic acid, Salicylicamide Acetaminophen, Lactic acid	200[b]
Saccharose, Thiourea	115
Hydroquinone	35

[a] Maximum weight ratio of foreign compounds to ascorbic acid 10 μg/mL giving an error of < 5%, [b] Maximum amount tested.

Application to Vitamin C tablets

The proposed method was applied to the determination of AsA in vitamin C tablets. Table 5 lists the results obtained on application of proposed method. These results are compared with those obtained with 2,6-dicholorophenolindophenol (DCPIP), and indicate that the proposed method is rapid and selective and could be readily implemented using a very simple and stable reagent delivery system.

Table 5. Determination of ascorbic acid in Vitamin C tablets, (mg/tablet).

Sample	Proposed method mean ± SD[a]	Standard method (DCPIP) mean ± SD[a]
Vitamin C tablet (1000mg)	996.0 ± 6.3	997.7 ± 9.0
Vitamin C tablet (500mg)	495.7 ± 5.3	498.1 ± 4.7
Vitamin C tablet (500mg), daily cold & Flu defence [b]	496.4 ± 3.7	498.4 ± 6.0
Vitamin C tablet (200mg), daily cold & Flu defence [b]	200.2± 2.1	199.1 ± 2.0
Vitamin C tablet (500mg)	494.0 ± 4.6	500.2 ± 3.8
Vitamin C tablet (500mg)	493.3 ± 5.0	498.0 ± 4.1
Vitamin C tablet (250mg)	246.5 ± 3.3	248.1 ± 3.8

[a] Standard deviation, [b] The Boots company PLC Nottingham England.

Conclusions

The FI method is rapid, simple, sensitive and accurate for ascorbic acid in pharmaceuticals and real samples. A negative peak results from an injection of ascorbic acid into an iron(III)-thiocyanate complex carrier stream. The method works satisfactorily in the presence of a wide variety of materials that are encountered in multi-component vitamin C formulations. The iron(III)-thiocyanate reagent used is inexpensive, safe and readily available in most laboratories. The recovery for the proposed FIA system is close 100%.

Acknowledgements

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References

1. M. Kunkel, T. Meyer, J. Bohler, A. W. Farham, *J. Pharm. Biomed. Anal* **1999**, *21*, 911–922.
2. J. Ren, H. Zhang, Q. Ren, C. Xia, J. Wan, Z. Qin, *J. Electroanal. Chem.* **2001**, *504*, 59–63.
3. J.-M. Zen, D.-M. Tsai, A. S. Kumar, V. Dharuman, *Electrochem. Comm.* **2000**, *2*, 782–785.
4. X. Han, J. Tang, J. Wang, E. Wang, *Electrochem. Acta* **2001**, *49*, 3367–3371.
5. J. Wang, Z. Wu, J. Tang, R. Teng, E. Wang, *Electroanalysis* **2001**, *13*, 1093–1097.
6. P. Wang, X. Wang, X. Jing, G. Zhu, *Anal. Chim. Acta* **2000**, *424*, 51–56.
7. H. Iwase, I. Ono, *J. Chromatogr. A*. **1988**, *806*, 361–364.
8. S. P. Sood, L. E. Sartori, D. P. Wittmer, W. G. Haney, *Anal. Chem.* **1976**, *48*, 796–798.
9. M. Noroozifar, M. Khorasani-Motlagh, *Talanta*, **2003**, *61*, 173–179.
10. Z.-Q. Zhang, Y.-C. Jiang, *Atom. Spec.* **2000**, *21*, 100–104.
11. M. C. Yebra, R. M. Cespón, A. Moreno-Cid, *Anal. Chim. Acta* **2001**, *448*, 157–164.
12. Y.-C. Jiang, Z.-Q. Zhang, J. Zhang, *Anal. Chim. Acta* **2001**, *435*, 351–355.

13. A. V. Pereira, O. Fatibello-Filho, *Anal. Chim. Acta* **1988**, *366*, 55–62.
14. D. G. Themelis, P. D. Tzanavaras, F. S. Kika, *Talanta* **2001**, *55*, 127–134.
15. A. Paula, S. Paim, B. F. Reis, *Anal. Sci.* **2000**, *16*, 487–491.
16. A. Paula, S. Paim, C. M. N. V. Almeida, B. F. Reis, R. A. S. Lapa, E. A. G. Zagatto, J. L. F. Costa Lima, *J. Pharm. Biomed. Anal.* **2002**, *28*, 1221–1225.
17. M. A. Raggi, V. Cavrini, A. M. Dipietra, *J. Pharm. Sci.* **1982**, *71*, 1384–1386.
18. M. N. Reddy, D. G. Sankar, V. V. Sekhar, N. Ravindra, G. D. Rao, *Indian Drugs* **1991**, *28*, 331–333.
19. L. Fu, Y. Ren, *Fenxi Huaxue* **1992**, *20*, 193–195.
20. P. B. Issopoulos, P. T. Economou, *Anal. Chim. Acta* **1992**, *257*, 203–207.
21. F. Buhl, U. Hachula, *Chem. Anal. (Warsaw)* **1984**, *29*, 483–489.
22. J. Bassett, R. C. Denney, G. H. Jeffery, J. Mendham, *Vogel's Textbook of Quantitative Inorganic Analysis;* 4th ed., Longman Scientific & Technical, London and New York, 1978, pp.741.
23. N. K. Pandey, *Anal. Chem.* **1982**, *54*, 793–798.
24. W. Horwitz, *Official Methods of Analysis of the Association of Official Analytical Chemists;* Published by the Association of Official Analytical Chemists, Washington DC, 3rd ed., 1980, pp.746.
25. D. A. Skoog, D. M. West, F. J. Holler, *Fundamental of Analytical Chemistry;* Saunders College Publishing, New York, 5th ed., 1988, chapter 7.
26. K. Ozutsumi, M. Kurihara, T. Miyazawa, T. Kawashima, *Anal. Sci.* **1992**, *8*, 521–526.

Povzetek

Razvita je bila enostavna spektrometrična metoda za posredno določevanje L-askorbinske kisline s pretočno injekcijsko analizo na osnovi redukcije Fe(III)-tiocianatnega komleksa v kislem mediju. Ob injiciranju L-askorbinske kisline v nosilno raztopino, ki vsebuje Fe(III)-tiocianatni komleks, se pri kontinuirni meritvi pojavi negativni vrh zaradi znižanja absorbance merjene pri 462 nm. Velikost negativnega vrha je sorazmerna koncentraciji L-askorbinske kisline v vzorcu. Metoda zagotavlja mejo detekcije 0,36 μg/mL, linearno območje meritev do 100 μg/mL in standardni odmik 2,0% (n=6). Pri 95% stopnji zaupanja ni bolo opaženih razlik v primerjavi z rezultati, ki jih daje ročna metoda. Opisani sistem omogoča določevanje L-askorbinske kisline v farmacevtskih preparatih in živilih.