LRA, Vol. 11, pp. 45–50

Flow-Injection Spectrophotometric Determination of Vitamin B_1 (Thiamine) in Multivitamin Preparations

Clezio Aniceto, Airton Vicente Pereira, Cícero Oliveira Costa-Neto, and Orlando Fatibello-Filho

Departamento de Química, Grupo de Química Analítica, Centro de Ciências Exatas e de Tecnologia, Universidade Federal de São Carlos, Caixa Postal 676, CEP 13.560-970—São Carlos, SP, Brazil; E-mail: bello@dq.ufscar.br

Accepted 19 August 1999

ABSTRACT: A flow-injection (FI) spectrophotometric procedure is proposed for the determination of vitamin B1 (thiamine hydrochloride) in multivitamin preparations. Powdered sample, containing from 25 to 100 mg of multivitamin preparations, was previous dissolved in 0.1 mol L^{-1} hydrochloric acid, and a volume of 250 μL was injected directly into a carrier stream of 0.10% (w/v) potassium hexacyanoferrate(III) in 0.5 mol L^{-1} sodium hydroxide at a flow rate of 2.46 mL min^{-1} an FI system. The thiochrome produced in the oxidation of thiamine hydrochloride by potassium hexacyanoferrate(III) in alkaline solution was directly measured at 369 nm. Potassium hexacyanoferrate(III) in this concentration did not cause any interference.

Vitamin B_1 was determined in three multivitamin preparations in the concentration range from 2.5 to 50.0 mg L^{-1} (calibration graph: $A = -0.0132 + 0.0134\ C$, $r = 0.9990$, where A is the absorbance and C is the vitamin B_1 concentration in mg L^{-1}). Sucrose, glucose, fructose, lactose, citric acid, starch, vitamin B_2, and vitamin B_6 do not interfere, even in concentrations five times higher than vitamin B_1. Only vitamin B_{12} causes interference, but this vitamin is not present in the multivitamin preparations used in this work. The detection limit was 1.0 mg L^{-1}, and the recovery of vitamin B_1 from three samples ranged from 97.5 to 105.0. The sampling rate was 41 h^{-1} and RSDs were less than 1% for solutions containing 10.0 and 30.0 mg L^{-1} vitamin B_1 ($n = 10$). The results obtained for the determination of vitamin B_1 in commercial preparations are in good agreement with those obtained by differential pulse polarography ($r = 0.9999$) and also with the label values ($r = 0.9998$).
 Lab Robotics and Automation 11: 45–50, 1999

Correspondence to: Orlando Fatibello-Filho.
Contract grant sponsor: FAPESP; contract grant numbers: 91/2637-5; 1994/4822-2.
Contract grant sponsor: PADCT/CNPq; contract grant number: 62.0060/91-3.
Contract grant sponsor: CNPq; contract grant number: 50.1638/91-1.
Laboratory Robotics and Automation
 CCC 0895-7533/99/010045-06

INTRODUCTION

Vitamin B_1 (thiamine hydrochloride) is a white crystalline powder, hygroscopic, with a yeasty odor and

salty, nutlike taste [1]. A deficiency of this vitamin in the diet results in the disease known as beriberi [2, 3]. Because of the sensitivity of thiamin hydrochloride to adverse conditions such as light, heat, and neutral and alkaline pHs, proper precautions should be taken to prevent any deterioration throughout the analytical process. There are a plethora of bioassay, microbiological, and chemical methods for the determination of vitamin B_1 in a variety of samples described in the literature [1–21]. Bioassay and microbiological methods are cumbersome and time-consuming [4, 5], while chemical methods offer great accuracy and speed [6]. The chemical method most commonly used involves oxidation of thiamine with potassium hexacyanoferrate(III) producing thiochrome, which is then measured fluorimetrically. This procedure is the official AOAC method [7]. Nevertheless, the thiochrome procedure requires a skilled technician, and the analytical steps such as oxidation of thiamine, solvent extraction, and centrifugation must be performed under subdued light because both thiamine and thiochrome are light sensitive. Also, the fluorescence measurement of thiochrome in the organic medium needs to be carried out rapidly and precisely according to the instructions; otherwise, inconsistent results could be obtained. The thiochrome method has been adapted in a flow-injection (FI) procedure with fluorescence [8] and chemiluminescence [9] measurements. While in the form procedure [10], the flow rates of organic streams (chloroform) change because of mechanical deterioration of the Acidflex tubings, leading for this reason to the need to use three standard solutions after each set of not more than six sample solutions, the latter has low sensitivity and also needs frequent recalibrations due to the intrinsic chemiluminescence analyzer characteristics. Another FI spectrofluorimetric procedure, the potassium hexacyanoferrate(III), was replaced by mercury(II), but the waste solution from this FI system should receive a special treatment so as not to cause environmental problems [11]. Finally, an in situ photochemical reaction of thiamine sensitized by acetone has also been proposed [12]. Thiamine, a nonfluorescent compound, is converted in alkaline medium by the sensitized photochemical reaction into an intensively fluorescent compound, which has the same characteristic maxima of fluorescence excitation and emission spectra as those of thiochrome. Using an organic solvent such as acetone and the peristaltic pump should be stopped for at least 60 seconds when the sample zone is in the flow cell to open the light shutter to irradiate the solution at an excitation wavelength of 280 nm and emission at 440 nm. As it is well known, organic solvent can speed up the damage of the flow tubing and can generate bubbles in the system, due to the decreasing of oxygen solubility. Surprisingly, any FI spectrophotometric procedure for determining thiamine was found in the literature.

In this work, an FI spectrophotometric procedure is proposed for determining vitamin B1 (thiamine hydrochloride) in multivitamin preparations without the need of organic phase separation, previously the spectrophotometric or fluorimetric detection. The thiochrome produced in the oxidation of thiamine hydrochloride by potassium hexacyanoferrate(III) in alkaline solution was directly measured at 369 nm (Figure 1). The results of FI conditions, interference study, and application of this procedure to the determination of thiamine in different samples are reported.

EXPERIMENTAL

Apparatus

A Hewlett-Packard (Boise, ID) Model 8452 A UV-visible spectrophotometer was used in the preliminary spectrophotometric investigations.

All spectrophotometric measurements were carried out in a Femto (São Paulo, Brazil) Model 435 spectrophotometer with a quartz cell (optical path 1.0 cm) connected to a Cole Parmer (Niles, IL) Model 12020000 two-channel strip-chart recorder. An eight-channel Ismatec (Zurich, Switzerland) Model 7618-40 peristaltic pump supplied with Tygon tubing was used for the propulsion of the fluids. The manifold employed was constructed with Tygon tubing (0.8

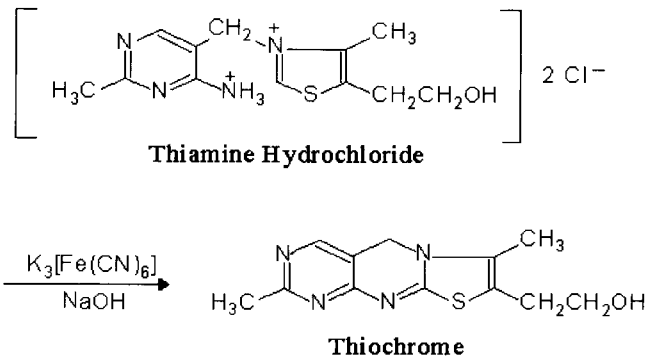

Figure 1. Scheme of the oxidation reaction of the thiamine hydrochloride to thiochrome by potassium hexacyanoferrate(III) in alkaline solution.

mm id). Sample and reference solutions were introduced in the flow system with the aid of a Micronal automatic proportional unity (model B352, São Paulo, SP, Brazil). The electronic time control of this device was not employed in the performed experiments.

All differential pulse polarographic measurements were performed with a Multi-Function Routine Polarograph Radelkis (Budapest, Hungary) Model OH-107.

Reagents and Solutions

Thiamine hydrochloride (vitamin B_1), pyridoxine hydrochloride (vitamin B_6), and cyanocobalamine (vitamin B_{12}) were biochemical-grade reagents. All other reagents were of analytical grade, and water from a Millipore (Bedford, MA) Milli-Q system (Model UV Plus Ultra-Low Organics Water) was used. Sucrose, glucose, fructose, lactose, citric acid, and starch were purchased from Sigma Chemical Co. (St. Louis, MO). A 100 mg L^{-1} thiamine hydrochloride stock solution was prepared in a 0.1 mol L^{-1} hydrochloric acid. Vitamin B_1 reference solutions from 1.25 to 50.00 mg L^{-1} were prepared daily in 0.1 mol L^{-1} hydrochloric acid. All thiamine solutions were protected from light. Potassium hexacyanoferrate(III) (0.10% m/v) in 0.5 mol L^{-1} sodium hydroxide was prepared daily by dilution of a 1.0 % m/v potassium hexacyanoferrate(III) stock solution in 0.5 mol L^{-1} sodium hydroxide.

Sample Preparation

Vitamin B_1 was determined in Benerva and Complexo B (Roche Químicos e Farmacêuticos SA, Rio de Janeiro, RJ, Brazil) and Doxal (A Nova Química Laboratório SA, São Bernardo do Campo, SP, Brazil) multivitamin preparations.

Ten tablets were powdered, and an accurately weighed amount of 25–100 mg was transferred to a 100 mL beaker containing 25 mL of 0.1 mol L^{-1} hydrochloric acid and was stirred for 10 minutes. The solution was filtered through a Whatman No. 1 filter paper, and the filtrate and two washings, each of 5 mL, were collected in a 50 mL calibrated flask and diluted to volume with the same acid solution. Appropriate dilutions to produce solutions within the linear range of the calibration curve were used in the analysis.

Differential Pulse Polarography

The polarographic DC method proposed by Pleticha [13] was adapted to a differential pulse polarographic mode using multiple standard addition methodology for vitamin B_1 determination in multivitamin preparations.

Flow-Injection Procedure

A schematic diagram of the flow manifold is shown in Figure 2. The vitamin B_1 solution was introduced into the carrier stream with the aid of an injector commutator with a loop volume of 250 μL. A solution of 0.10% m/v potassium hexacyanoferrate(III) in 0.5 mol L^{-1} sodium hydroxide flowing at 2.46 mL min^{-1} was used as carrier. When a solution of vitamin B_1 is injected into the carrier stream, the thiochrome produced in the oxidation of thiamine hydrochloride by potassium hexacyanoferrate(III) in alkaline solution was directly measured at 369 nm, and it is proportional to the thiamine concentration.

RESULTS AND DISCUSSION

Preliminary Studies

The oxidation of thiamine with hexacyanoferrate(III) in alkaline medium involves the formation of thiochrome (Figure 1). A spectrophotometric study of the reaction of thiamine with potassium hexacyanoferrate(III) in 0.1 mol L^{-1} NaOH was carried out, and the absorption spectrum of the thiochrome produced after 1 min is shown in Figure 3. The absorption of the thiochrome did not increase significantly until 5 minutes. As can be seen, the thiochrome formed can de monitored spectrophotometrically by measuring the absorbance at 369. Potassium hexacyanofer-

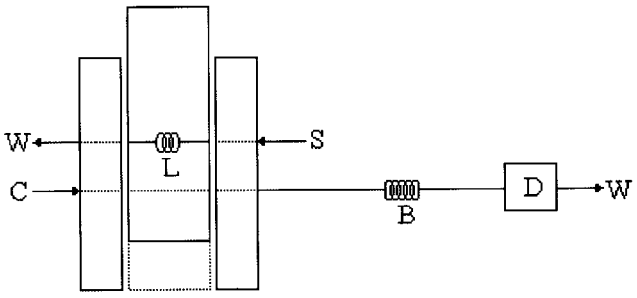

Figure 2. Schematic diagram of the flow system used for vitamin B_1 determination. Automatic proportional injector commutator shows the injection position after commutation. S, sample or standard solutions; L, sample loop (250 μL); C, carrier solution, 0.10% w/v potassium hexacyanoferrate(III) in 0.5 mol L^{-1} sodium hydroxide, flowing at 2.46 mL min^{-1}; D, spectrophotometer (λ = 369 nm); B, tubular coiled reactor (100 cm); W, waste.

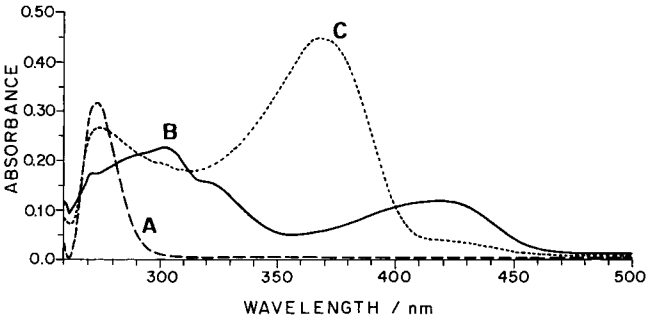

Figure 3. Spectra of 10 μg mL^{-1} thiamine hydrochloride (A), 0.10% (w/v) hexacyanoferrate(III) in 0.50 mol L^{-1} NaOH (B) and thiochrome (C) produced (after 1 min) in the reaction of thiamine with 0.10% (w/v) hexacyanoferrate(III) in 0.5 mol L^{-1} NaOH.

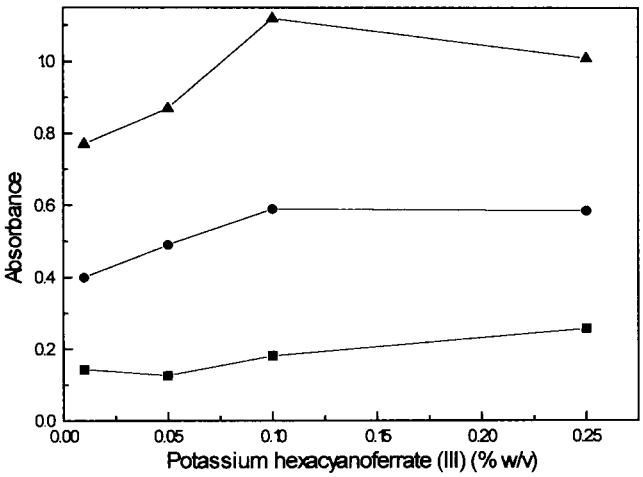

Figure 4. Effect of potassium hexacyanoferrate(III) concentration from 0.01 to 0.25% (w/v) used as carrier solutions on the analytical response for thiamine solutions in the concentrations of ■■, 5.0; ●●, 25.0; and ▲▲, 50.0 mg mL^{-1}. Others variables were as described in Figure 2.

rate(III) solution in this concentration did not cause any interference, and the use of both organic solvent and solvent extraction of the thiamine to an organic phase prior its spectrophotometric or fluorimetric determination was not necessary. Taking in account this spectral behavior, an FI procedure was developed, as shown in Figure 2.

Chemical and FI Parameters

Initially, the FI manifold was used to investigate the experimental conditions for the oxidation of thiamine with potassium hexacyanoferrate(III) in alkaline medium. In this experiment, the reactor coil length (B), sample volume (L), and carrier flow rate used were 50 cm, 250 μL, and 2.46 mL min^{-1}, respectively.

Figure 4 shows the effect of potassium hexacyanoferrate(III) concentration in the range from 0.01 to 0.25% (w/v) in 0.1 mol L^{-1} NaOH solution for thiamine solutions in the concentrations of 5.0, 25.0, and 50 mg mL^{-1} on the baseline, and the absorbance signal was studied. As can be observed from this figure, the absorbance signal increased with an increase in the potassium hexacyanoferrate(III) concentration used up to 0.10% w/v and then leveled off between 0.10 and 0.25% w/v potassium hexacyanoferrate. Therefore, a concentration of 0.10% w/v of this reagent was selected for further experiments.

The effect of the NaOH concentration varying from 0.25 to 2.0 mol L^{-1} in potassium hexacyanoferrate(III) solution on the thiamine oxidation was also evaluated (Figure 5). The absorbance signals measured in this NaOH concentration range showed that the absorbance signals increased up to 1.0 mol L^{-1} NaOH solution. A 0.5 mol L^{-1} NaOH was chosen because a best linearity and repeatability were obtained. Therefore, all further experiments were carried out with a 0.10% (w/v) potassium hexacyanoferrate(III) in 0.5 mol L^{-1} NaOH solution.

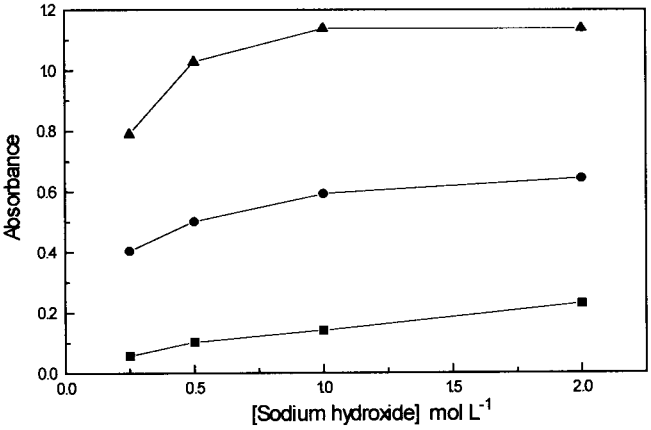

Figure 5. Effect of the NaOH concentration from 0.25 to 2.0 mol L^{-1} in the 0.10% (w/v) potassium hexacyanoferrate(III) solution used as carrier on the determination of thiamine (■■, 5.0; ●●, 25.0; and ▲▲, 50.0 mg mL^{-1}).

The effect of FI parameters such as carrier flow rate, sample volume, and coiled reactor length were investigated in order to attain the best compromise between sensitivity, repeatability, and sample throughput.

The carrier flow rate was investigated in the flow-rate range from 1.43 to 3.61 mL min^{-1} with a sample loop of 250 μL. Increasing the carrier flow rate of the 0.10% (w/v) potassium hexacyanoferrate(III) in 0.5

mol L^{-1} NaOH solution resulted in an increase in the absorbance of up to 2.46 mL min^{-1} and then a decrease in the signal slightly up to 3.61 mL min^{-1}. Consequently, a flow rate of 2.46 mL min^{-1} for the carrier solution was used.

The volumes of thiamine solutions injected varied between 125 and 375 μL (50 and 150 cm). The absorbance increased slightly with increasing volumes up to 375 μL of thiamine. Therefore, a sample volume of 125 μL (L, 50 cm) was selected in this work.

The effect of the reactor coil length (*B*) on the sensitivity of the FI method was studied in the range 75–200 cm. With respect to sensitivity and analytical rate, the best compromise was achieved by using a reactor length of 100 cm.

Interferences and Recovery Studies

The effect of several potential interferents on the determination of vitamin B_1 caused by common excipients used in tablets and other vitamins was evaluated by adding 50 mg L^{-1} solution of each one of these potential interferents (sucrose, glucose, cellulose, magnesium stearate, lactose, starch, riboflavin, pyridoxine hydrochloride, and cianocobalamine) into a pure solution of the vitamin B_1. The presence of fivefold excess amounts of these compounds did not cause any influence in the determination of vitamin B_1 in the measurement conditions used. The most severe interference was observed with samples containing riboflavin, which is typically yellow, that absorbs light in the same wavelength region as the thiochrome. However, this vitamin is not present in the multivitamin preparations used in this work.

An addition-recovery experiment was carried out by spiking the real samples with three different thiamine reference solution concentrations (2.0, 4.0, and 8.0 mg L^{-1}). Recoveries of 97.5–105.0% of thiamine were obtained using the proposed FI spectrophotometric method.

Calibration Graph and Applications

A series of vitamin reference or sample solutions was injected in triplicate into the manifold depicted in Figure 2. The transient signals for nine thiamine reference solutions and triplicate signals for three pharmaceutical preparation solutions (Figure 6) demonstrate good precision and baseline stability. The calibration graph for thiamine was linear in the concentration range from 2.5 to 50.0 mg L^{-1} ($A = -0.0132 + 0.0134\ C$; $r = 0.9990$, where A is the absorbance and C the concentration of vitamin B_1 in mg L^{-1}). The detection limit (three times the signal blank/slope) of the FI procedure was 1.0 mg L^{-1} vitamin B_1. The results obtained using the FI proposed procedure were compared with those results obtained by a differential pulse polarographic method ($r_1 = 0.9999$) and also with those declared on the labels ($r_2 = 0.9998$) (Table 2). The precision of the FI spectrophotometric method was tested by repeated runs ($n = 10$) of 10.0 and 30 mg L^{-1} of vitamin B_1 solutions. The relative standard deviations were lower than 1.0%, and the analytical frequency was 41 h^{-1}.

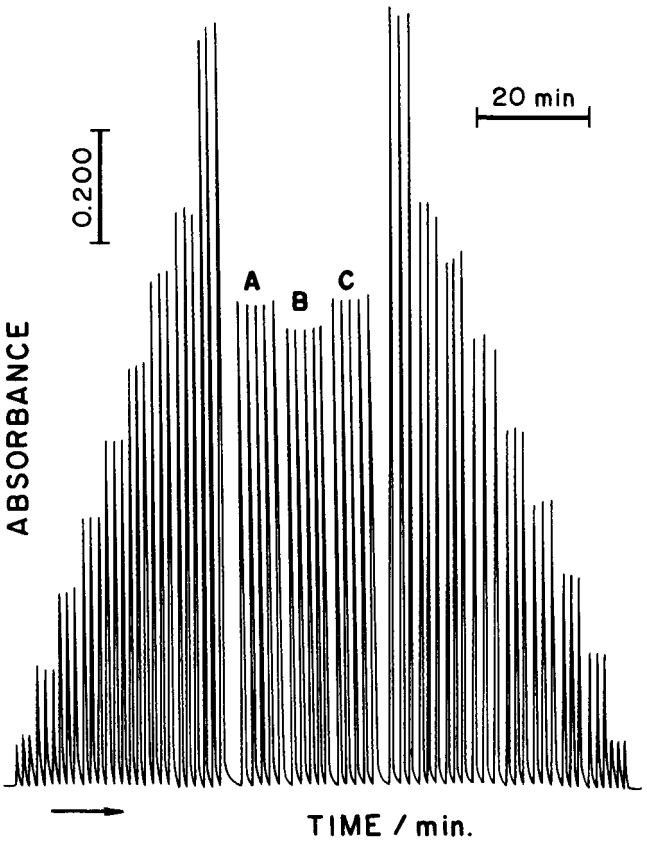

Figure 6. Transient absorbance signals obtained in triplicate for vitamin B_1 reference (thiamine hydrochloride) in 0.1 mol L^{-1} hydrochloric acid solutions from 2.5 to 50.0 mg L^{-1} and pharmaceutical preparations. From left to right 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, and 50.0 mg L^{-1} of vitamin B_1 reference solutions and three pharmaceutical preparations (A, Benerva; B, Doxal; C, Complexo B) and reference solutions therein.

CONCLUSION

An FI method proposed in this work for the determination of thiamine in pharmaceutical preparations

TABLE 1. Results of Addition-Recovery Experiment Using Thiamine with Three Different Standard Concentrations

Sample	Thiamine (mg L^{-1}) Added	Thiamine (mg L^{-1}) Recovered	Recovery (%) ($n = 4$)
Benerva	2.0	2.0	100.0
	4.0	3.9	97.5
	8.0	7.9	98.8
Complexo B	2.0	2.0	100.0
	4.0	4.1	102.5
	8.0	8.4	105.0
Doxal	2.0	1.9	98.5
	4.0	4.1	102.5
	8.0	8.2	102.5

TABLE 2. Thiamine Determination in Multivitamin Preparations by Using the Differential Pulse Polarography and the Flow-Injection Spectrophotometric Procedures

Sample	Thiamine (mg tablet) Label	Polarographic	FIA*	Relative Error (%) E_1	E_2	CV %
Benerva	300	298.0 ± 1.4	302.4 ± 1.1	+0.8	+1.5	0.4
Complexo B	5	5.1 ± 0.5	5.0 ± 0.1	0.0	−2.0	2.0
Doxal	30	30.4 ± 0.2	31.5 ± 0.3	+5.0	+3.6	0.9

E_1 = FIA spectrophotometric versus label value; E_2 = FIA spectrophotometric versus polarographic.
*$n = 6$, confidence level 95%.

based on the absorbance measurement of thiochrome produced in the oxidation of vitamin B_1 (thiamine) by potassium hexacyanoferrate(III) in alkaline solution is simple, precise, accurate, and involves no sample preparation such as organic solvent or solvent extraction of thiamine to an organic phase prior to its determination. The good correlation between the results obtained using the FI spectrophotometric proposed procedure and those obtained by a differential pulse polarographic method suggests that the FI procedure may be suitable for routine analysis.

ACKNOWLEDGMENTS

The scholarships granted by CNPq to C.A., A.V.P., and C.O.C.N. are gratefully acknowledged.

REFERENCES

[1] Augustin, J. In Introduction to the Chemical Analysis of Foods; Nielsen, S. S., Ed.; Jones and Bartlett: Boston, 1994; pp 251–260.

[2] Korolkovas, A.; Burckhalter, J. H. Química Farmacêutica, 1st ed.; Guanabara Dois: Rio de Janeiro, 1982; pp 575–668.

[3] Schanderl, S. H. In Methods in Food Analysis; Joslyn, M. A., Ed.; Academic: New York, 1970; pp 745–791.

[4] Kozik, A. J Biochem Biophys Meth 1994, 28(2), 147.

[5] Herve, C.; Beyne, P.; Delacoux, E. J Chromatog Biomed Appl 1994, 653(2), 217.

[6] Fang, G. Z.; Chen, M.; Peng, M.; Yang, L. Z. Yaowu Fenxi Zazhi 1994, 14(2), 19.

[7] Williams, S. Official Methods of Analysis; 40[a] ed.; AOAC: Arlington, VA, 1984; pp 813–829, 832–834, 861–864.

[8] Jie, N.; Yang, J.; Zhan, Z. Anal Lett 1993, 26(10), 2283.

[9] Grekas, N.; Calokerinos, A. Talanta 1990, 37(11), 1043.

[10] Kalberg, B.; Thelander, S. Anal Chim Acta 1980, 114, 129.

[11] Lozano, C. M.; Ruiz, T. P.; Tomás, V.; Abellán, C. Analyst 1990, 115, 217.

[12] Guo, X. Q.; Xu, J. G.; Wu, Y. B. Z.; Huang, X. Z.; Chen, G. Z. Anal Chim Acta 1960, 276, 151.

[13] Pleticha, R. Pharmazie 1957, 12, 219.

[14] Huang, B.; Yu, B.; Li, P. B.; Jiang, M.; Bi, Y. S.; Wu, S. F. Anal Chim Acta 1995, 312(3), 329.

[15] Hart, J. P.; Norman, M. D.; Tsang, S. Analyst 1995, 120(4), 1059.

[16] Stroheccker, R.; Henning, H. M. In Analisis de Vitaminas; Mayor, F., Ed.; Paz Montalvo: Madrid, 1967.

[17] Wang, Q. L.; Chen, M. T.; Lihua-Jianyan, Huaxue-Fence 1995, 31(5), 297.

[18] Barna, E.; Dworschak, E. J Chromatog 1994, 668(2), 359.

[19] Ollainen, V.; Vahteristo, L.; Uusi-Ranva, A.; Varo, P.; Koivistoinen, P.; Huttunen, J. J Food Compos Anal 1993, 6(2), 152.

[20] Yu, X. J.; Qu, X. G.; Lu, T. H.; Dong, S. J. Fenxi-Huaxue 1994, 22(11), 1111.

[21] Garcia, F. P.; Díaz, C.; Hardisson, A. Anal Lett 1993, 26(9), 1977.