PERIODICO di MINERALOGIA Volume 90, No. 6, 2021

Determination of vitamin C in pharmaceutical products with physico-chemical and bioanalytical technics

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Abstract

Vitamin C is an important biological molecule involved in many biological and biochemical processes as reducing agent, nutritional factor and enzyme cofactor. A large number of methods have been developed for quantifying vitamin C contents in pharmaceuticals. It is therefore essential to assess these methods. The present paper reviews all these methods.

Keywords: vitamin C, ascorbic acid, pharmaceuticals analysis, spectrophotometry, biosensor.

Introduction

The deficiency of this vitamin leads to many diseases : scurvy, plug poisoning, liver disease, allergic reactions, arteriosclerosis, etc. Other symptoms of its deficiency have been reported, but they are not well defined. It is believed to participate in intermediate metabolism besides its involvement in the immune system, biosynthesis and metabolism of certain compounds. Also, it has been identified as a radical scavenger in vivo. Keeping in view its importance, the analysis of pharmaceuticals containing this vitamin assumes significance.

The determination of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical and food application.

A large number of methods have been reported for the determination of acid ascorbic: titrimetry, voltammetry, potentiometry, fluorometry, spectrophotometry, kinetic-based chemiluminescence, flow injection analyses (FIA) and chromatography.

There are numerous non-spectrophotometric methods, which have been reviewed by Arya [1] recently and their number and kind are increasing rapidly.

Results and Discussions

1. Titrimetric methods

It have been reported many titrimetric methods using different titrants.

Determination of acid ascorbic with iodine, potassium iodate and potassium bromate and iodine monochloride [2,3] using starch as an indicator has been reported by many workers, but it was observed that starch cannot be used in such titrations because it decreases the reaction rate between ascorbic acid and iodine. Some other reagents such as variamine blue, carbon tetrachloride or chloroform in the presence of mercuric chloride and p-

ethoxychrysoidine as indicators have been recommended. Their use in ascorbic acid assay has been reviewed by Murty and Rao [4] who proposed naphthol blue black, amaranth or Brilliant Ponceau 5R as alternative indicators.

A recent oxidimetric method [5] involves the use of chloramine-T for ascorbic acid mixtures with thiols. This method requires the masking of thiol by cyanoethylating it with acrylonitrile. The authors claim that the cyanoethylated products of thiols do not hamper the reaction and that ascorbic acid could be titrated with chloramine-T using, 2,6-dichlorophenolindophenol as indicator. This titrimetric finishing step is based on the method [6] of determining several oxidizing agents including chloramine-T by titrating with ascorbic acid using 2,6-dichlorophenolindophenol as indicator.

2,6-Dichlorophenolindophenol (DCIP) has been used as the titrant for the determination of acid ascorbic [7]. The method is based on the reduction of DCIP with acid ascorbic in acidic solution. It is an official method [8], it is not applicable to many pharmaceutical preparations containing Fe(II), Sn(II), Cu(I), SO_2, SO_3^{2-} and $S_2O_3^{2-}$ ions which are usually associated with mineral preparations. The applicability of the method is restricted to only those multivitamin tablets which do not contain minerals.

Moreover, DCIP has been found to oxidize thiols [9,10] and the mechanism of this reaction has also been explored in detail [11,12]. Consequently, a mild oxidizing agent like DCIP (redox potential +0.217) or any other oxidizing agent could not be used for the determination of ascorbic acid in the presence of thiols. On a project on the determination of sulfur compounds (13,14) it was found that tetrachlorobenzoquinone reacts stoichiometrically with ascorbic acid but does not react at all with strong reducing agents like thiols under identical conditions.

Resolutions of mixtures of vitamin C with thiols has been successfully carried out by first titrating the vitamin C content with tetrachlorobenzoquinone till the orange-red color appears. Upon dilution of the contents, thiols can be titrated with standard chloramine-T solution. Thiols are quantitatively oxidized to their corresponding disulfides with chloramine-T in the presence of potassium iodide [15]. The method gives excellent results in mixtures and pharmaceutical preparations (Suckcee - IDPL, Cilin - Glaxo, Chewcee - Lederle, Cecon - Abbot, Redoxon - Roche).

$$CH_3C_6H_4SO_2NCl^-Na^+ + 2I^- + 2H^+ \rightarrow CH_3C_6H_4SO_2NH_2 + Cl^-Na^+ + I_2$$

$$2RSH + I_2 \rightarrow RSSR + 2HI$$

N-bromosuccinimida was introduced as a reagent for the determination of ascorbic acid using starch as an indicator. Reductones, reductic acid and iron salts do not interfere with this titration, but it was found to give unsatisfactory results. Quinoline yellow solution has been used for detecting the end point in the titrations with N-bromophthalimide and N-bromosaccharin [16]. These reagents were also used in the potentiometric titration of vitamin C. Chauhan and Singh [17] made use of N-bromosaccharin for the microdetermination of ascorbic acid in pharmaceutical preparations.

Although the titrimetric methods are simple to use, difficulties are encountered even with commonly used titrants. Additional problems are met especially with colored samples or in the presence of reducing substances which can bleach the dye and make the analysis non-specific. Edible dyes such as amaranth, indigotine or tetrazine present in pharmaceuticals need removal either by ion exchange or by addition of charcoal. Such limitations have encouraged chemists to look for better alternative methods.

2. Electrochemical methods

These methods play an important role in pharmaceutical analysis [18]. Application of some method depends on specific analytical-pharmaceutical problem. Voltammetry [19-23] is

very popular in this field, because of high sensitivity, selectivity and linear response in low concentration range. If content of the active species of drug is large a constant potential coulometry was successfully applied [24]. In addition to the voltammetric techniques a controlled current coulometry (coulometric titration) can be used for analysis of drug in dosage form. An electric standard is used in this technique, therefore, it is considered as a calibrationless method. If the content of determined compound is higher it involves high concentration of precursor from which titrant is generated at relatively long analysis times. High generation current can lead to lowering the current efficiency.

Similar is the principle of method presented by Tomcik [25]. It utilizes a fast and quantitative chemical reaction of analyzed species with electrogenerated reactant proceeding only in thin layer adjacent to the electrode surface (diffusion layer). Titrant is generated galvanostatically or with galvanostatic scan of small scan rate. The tablets of pharmaceuticals Antabus (Alpharma, NOR) and Celaskon (Leciva, CZE) served as the real samples.

Polarographic analysis involving electrochemical oxidation of L-ascorbic acid on a dropping mercury electrode has been employed for its determination in pharmaceuticals. The interference from reductones requires pretreatment of the sample with formaldehyde.

Voltammetric analysis using different electrodes (conventional electrodes [26-28], microdisc electrodes [29], microband and multiple microband electrodes [30] and carbon paste electrodes [31]) has been put to use for the determination of vitamin C. But the reliability of the general applicability of such electrodes decreases with repeated use because of electrode fouling by oxidation products. Pournaghi-Azar [32] reported the analysis of pharmaceutical preparations involving electrocatalytic oxidation of ascorbic acid in homogenous solutions using electrogenerated ferriciniumcarboxylic acid as a mediator.

In pharmaceutical tablets, vitamin C has been determined by differential pulse polarography [33-38] after derivatization with o-phenylene diamine in 0.2M acetate. Pharmaceuticals have been analyzed biamperometrically by titrating with iodine monochloride [39], potassium peroxomonosulfate [40], copper(II) perchlorate [41] or potassium iodate [42], using a platinum, wax-impregnated graphite electrode or a twin graphite electrode assembly [43]. Sun [44] suggested another method using electrochemical co-polymerization of 3,4-dihydroxybenzoic acid and aniline carried out at a microdisc gold electrode. Strohl and Curran [45] reported for the first time the use of a reticulated glassy carbon electrode as an amperometric flow-through detector in the flow injection determination of vitamin C. Amperometric flow injection methods [46-51] using immobilized enzyme reactors [46-48] or photochemical reduction of methylene blue [49] have also been recommended for the assay of ascorbic acid in 0.1M phthalate buffer (pH 3.8). The methylene blue method allows the determination of ascorbic acid in the range 5.0-90.0 μg/ml with a relative standard deviation of 1.3-4.8%.

Potentiometric titrations [51-57] have been employed for the determination of ascorbic acid using DCIP, N-bromosuccinimide, copper sulfate, iodine, potassium hexacyanoferrate(III), tetrachlorobenzoquinone and hexamminecobalt(III) triscarbonatocobaltate as titrants. The method reported by Nasser [55] is useful only for drugs without additives. Also, it lacks accuracy in the analysis of multivitamin formulations containing minerals or other oxidising compounds. Titrations involving hexacyanoferrate(III) are to be carried out in 4.5-5.5M sulfuric acid medium under an inert atmosphere and in 10-12M phosphoric acid. Microdetermination of L-ascorbic acid based on its oxidation by iodine in chloroform [53] or methanol [57] has also been carried out, which involves the determination of iodide ion formed with an iodide ion selective electrode; sulfide ion is expected to interfere. Some potentiometric methods using electrodes such as carbon paste [58], modified carbon paste [59], carbon fibre coated with the electrodeposited cobalt chelate

of tetra-N-methylpyridoporphyrazino cobalt [60] or a derivative f Co(II)-phtalocyanine doped with iodine [61] have been reported for the assay of ascorbic acid. In such titrations, the electrode can be fouled by proteins or other organic solutes leading to sluggish and drifting responses.

Coulometry, though very limited in its applications, has a few references in the determination of vitamin C. Coulometric methods based on the quantitative oxidation of ascorbic acid at a platinum anode have been investigated but only with pure solutions.

Other methods [62,63] involving the oxidation of ascorbic acid by iodine generated anodically are used in the analysis of pharmaceutical products.

3. Enzymatic methods

Uchiyama [64] have used a porous carbon felt electrode and hexacyanoferrate(III) ion as a mediator for the determination of L-ascorbate by rapid coulometry involving L-ascorbate oxidase.

A biosensor based on carbon paste modified with crude extract of zucchini (*Curcubita pepo*) as a source of peroxidase is proposed for determining L-ascorbic acid in pharmaceutical formulations (Cebion, Redoxon, Energil C, Vitamina C, Aspirina C) [65]. This enzyme in the presence of hydrogen peroxide catalyses the oxidation of hydroquinone to p-quinone whose electrochemical reduction back to hydroquinone was obtained at peak potential of –0.14V. Thus, when L-ascorbic acid is added to the solution, this acid can reduce chemically p-quinone to hydroquinone and/or reduce hydrogen peroxide, decreasing the peak current obtained proportionally to the increase of its concentration. The results obtained for L-ascorbic acid in pharmaceutical formulations using the proposed biosensor and those obtained using the Pharmacopeia method are in agreement at the 95% confidence level (**Table 1**).

Table 1. Determination of L-ascorbic acid in pharmaceutical formulations using the official method[66] and the biosensor

L-ascorbic acid (g/tablet)				
Sample	**Label value**	**Official method**	**Biosensor**	**Relativ error (%)**
Cebion	2.0	2.08±0.08	2.10±0.04	+ 1.0
Redoxon	1.0	1.09±0.07	1.05±0.03	- 3.7
Energil C	1.0	0.98±0.07	1.01±0.03	+ 3.1
Vitamina C	0.50	0.52±0.06	0.51±0.02	- 1.9
Aspirina C	0.24	0.24±0.05	0.25±0.02	+ 4.1

4. Chemiluminescent methods

A highly sensitive CL [67] procedure for the determination of ascorbic acid (1×10^{-9}-1×10^{-6}gml^{-1}) in tablets and vegetables with reagent flow injection analysis (FIA) is reported based on the inhibition of the CL reaction of the luminol-Fe^{2+}-O_2 system by ascorbic acid. Yet another flow injection CL method based on the sensitized photo-oxidation of ascorbic acid was proposed by Perez-Ruiz [68] for its determination in the range 1×10^{-9}-3×10^{-4}M. CL techniques are potentially very sensitive but require the use of a modified spectrophotometer for making measurements. Moreover, the lack of selectivity limits their direct application to the analysis of real samples.

5. Kinetic methods

Kinetic spectrophotometric methods with or without the application of stopped flow have been proposed using the reducing effect of ascorbic acid on DCIP [69-71] (λ_{max}=522nm)

or toluidine blue [72] (λ_{max}=600nm). However, these methods are time-consuming as they take at least 20min for one determination.

Several methods based on the reduction of iron(III) and measurement of the reduced iron(II) by its complexation with 1,10-phenanthroline [73-77], catalyzed light emission from luminol [78], oxidation of ascorbic acid by thalium(I) [79] and photochemical reduction of methylene blue [80-82] have been used for the analysis of drug formulations and fresh fruit juices. The interference of sulfur-containing reducing substances remains unchanged. Using a carrier stream of 2-mercaptoethanol [83,84], the difference in absorbance behavior of ascorbic acid and dehydroascorbic acid has been exploited in the determination of the former in pharmaceuticals by flow injection spectrophotometry.

6. Fluorometry methods

Lin [85] has described a facile and rapid method for the quantification of ascorbic acid in pharmaceutical preparations. The principle of the method is based on the observation that the fluorescence generated by xanthurenic acid (XA) itself can be proportionally quenched by ascorbic acid in a concentration-dependent manner. Owing to the stability of XA in acidic potassium phosphate buffer, a bulky reagent stock can be prepared and stored away for a long-term usage. In comparison with other method, this procedure is considerably faster and simpler because of the number of procedural steps is minimized, and thus the turn around time should be improved greatly.

Ascorbic acid, by oxidation with 2-hydroxynaphthaldehyde thiosemicarbazone [86] in the presence of Mn(II) catalyst or by its activating effect [87] on the oxidation of rhodamine 6G by potassium bromate in the presence of vanadium has been determined in pharmaceuticals by kinetic fluorometry.

The photo-oxidation of ascorbic acid sensitized with thionine blue was investigated by Perez-Ruiz [88] for its determination (8×10^{-7}-5×10^{-5}mol l^{-1}) in pharmaceuticals. Fluorometric methods that do not lead to the direct determination of vitamin C instead require calculations involving the correction of peak heights for blanks. Rigid control of pH is required in many of these methods since the intensity of the fluorescence of many substances is dependent on the pH of the solution.

7. Spectrophotometric methods

The majority of these methods are based on their oxidation-reduction properties or ability to couple with diazotized aniline derivatives. Many spectrophotometric methods based on the reduction of iron(III) to iron(II) with ascorbic acid, followed by complexation of reduced iron(II) with different reagents, such as 1,10-phenanthroline and 2,2'-dipyridyl, have been reported. On the other hand, acid ascorbic has several donor atoms capable of metal complex formation and binding with zinc(II), manganese(II), cadmium(II), alkaline earth metals and so on.

A simple and highly sensitive spectrophotometric method for determination of acid ascorbic was established by using iron(III) and p-carboxyphenylfluorone (PCPF) in a cationic surfactant micellar medium [89]. The apparent molar absorptivity of the proposed method, which does not require an extraction procedure, was $2.05\times10^{6}dm^{3}mol^{-1}cm^{-1}$ at 655nm. The procedure was succesfully applied to assays of ascorbic acid in pharmaceutical preparations, the analytical results were satisfactory. It was suggested that the colored complex produced was a mixture of the iron(II)-PCPF (1:2) complex and the DHAA-iron(III)-PCPF (1:2:2) complex.

Disposable, plastic cuvettes with integrated sensor layer made of Prussian blue were used for spectrophotometric determination of vitamin C in pharmaceutical products (Vitamin

C, Cebion, Aspirin C, Upsarin C, Scorbolamid, Efferalgan C, Strepsils, Calcium C, Magnesium, Duovit, Supradyn, Biovital) and presented in (**Table 2**) [90]. Ascorbates cause discolouration of the layer due to its reduction to the Prussian white. The decrease of absorbance of the film measured at 720nm is used as the analytical signal. The test is selective for vitamin C and the results of pharmaceuticals analysis are comparable to those obtained using reference pharmacopeal method. This test is extremely cheap (the cost of one PB film coated cuvette is lower than $0.1) and simple in fabrication. The analytical procedure is simple and no additional reagents are necessary to the performance of the test.

Table 2. Analysis of pharmaceutical products containing vitamin C

Medicine	**Supplier**	**Main compounds (s)**	**Content of acid ascorbic**	
			Cuvette test	**Iodometry**
Vitamin C	Polfa	Ascorbic acid	97±2mg/tab	98±4mg/tab
Cebion	Merck	Ascorbic acid	98±5mg/tab	98±2mg/tab
Aspirin C	Bayer	Acetylsalicylic acid	223±2mg/tab	220±2mg/tab
Upsarin C	Upsa	Acetylsalicylic acid	185±2mg/tab	188±3mg/tab
Scorbolamid	Polpharma	Salicylamide	137±2mg/tab	139±2mg/tab
Efferalgan C	Upsa	Paracetamol	193±2mg/tab	195±4mg/tab
Strepsils	Boots	Glucose, flavors	96±5mg/tab	96±4mg/tab
Calcium C	Polfa	Calcium lactogluconate	173±2mg/tab	176±2mg/tab
Magnesium	Polfa	Magnesium ascorbate	483±9mg/tab	482±5mg/tab
Duovit	Krka	Vitamins	55±1mg/tab	55±1mg/tab
Supradyn	Roche	Vitamins, minerals	133±4mg/tab	135±2mg/tab
Biovital	Rhone-Poulenc	Vitamins, minerals	17±3mg/tab	18±2mg/tab

Another spectrophotometric method was proposed by Pandey [15]. The ascorbic acid was determined spectrophotometrically at 336nm, via a decrease in absorbance in 7×10^{-4}M tetrachlorobenzoquinone (chloranil) in 80% acetone-water (v/v) at room temperature. The proposed method was successfully applied to pharmaceutical preparations. Strong reducing agents including most of thiols and serine, glycine, alanine, citric, oxalic, tartaric acids, glucose, sucrose and maltose do not interfere, even when present up to a 10-15 molar excess of vitamin C. Hence resolution of mixtures of vitamin C and thiols is possible, eliminating the use of a masking agent for thiols in other method.

Conclusions

Numerous methods have been reported for the determination of vitamin C in pharmaceuticals and more are to come. Every reported method is shown to find application in the analysis of one or the other type of samples. The measurement of vitamin C content in blood is of importance as it reflects the body's vitamin C status, and therefore, requires methods for its analysis which can provide precise and accurate results without sacrificing their sensitivity and selectivity.

More attention needs to be directed to finding the solution of the fundamental question of what role vitamin C plays in different organelles.

References

1. S.P. Arya, M. Mahajan, P. Jain, *Analytica Chimica Acta* 417 (2000), 1 –14.
2. ******Farmacopeea Romana*, ed X, Editura Medicala, Bucuresti, 1993.
3. *British Pharmacopoeia*, 5th ed., HM Stationery Office, London, 1988.
4. N.K. Murty, K.R. Rao, *J. Ind. Chem. Soc.* 53 (1976) 532.
5. K.K. Verma, A.K. Gulati, *Anal. Chem.* 52 (1980) 24.
6. G. Svehla, L. Kolai, L. Erdy, *Anal. Chim. Acta* 29 (1963) 442.
7. L. Erdy, G. Svehla, *Chemist-Analyst* 52 (1963) 24.
8. *Indian Pharmacopoeia*, Photolithio Press, Faridabad, 1985, p. 49.
9. R.E. Basford, F.M. Heunnkens, *J. Am. Chem. Soc.* 77 (1955) 3873.
10. C.G.Overberger, P.V. Bosignore, *J. Am. Chem. Soc.* 80 (1958) 5431.
11. N.K. Pandey, K.K. Mishra, *Indian J. Chem.*, in press.
12. N.K. Pandey, K.K. Mishra, M.J. Kashyap, *Phosphorus Sulfur*, in press.
13. A. Srivastava, S. Bose, *Talanta* 24 (1977) 517.
14. A. Srivastava, *Talanta* 20 (1979) 917.
15. N.K. Pandey, *Anal. Chem.* 54 (1982) 793.
16. K.G. Kumar, P. Indrasenan, *Talanta* 37 (1990) 269.
17. R.P.S. Chauhan, U.B. Singh, *J. Ind. Chem. Soc.* 72 (1995) 355.
18. P.T. Kissinger, W.R. Heineman, *Laboratory Techniques in Electroanalytical Chemistry*, second ed., Marcel Dekker, New York, 1996, p. 769.
19. J.E. Page, *J. Pharm. Pharmacol.* 4 (1952) 1.
20. M.R. Smyth, W.F. Smyth, *Analyst* 103 (1978) 529.
21. W.F. Smyth, A.D. Woolfson, *J. Clin. Pharm. Ther.* 12 (1987) 117.
22. G.J. Patriarche, J.C. Viré, *Anal. Chim. Acta* 196 (1987) 193.
23. P.M. Bersier, *J. Pharm.Biomed. Anal.* 1 (1983) 475.
24. K.S.V. Santhanam, V.R. Krishnan, *Anal. Chem.* 33 (1961) 1493.
25. P. Tomcik, M. Krajcikova, D. Bustin, *Talanta* 55 (2001) 1065-1070.
26. P.M. Korash, A.G. Eving, R.L. Wilson, R.M. Wightman, *J. Neurosci. Methods* 10 (1984) 215.
27. A.G. Fogg, A.M. Summan, *Analyst* 108 (1983) 691.
28. S. Kozar, et al., *Fresenius Z. Anal. Chem.*329 (1988) 760.
29. D.H. Carston, C.P. Jones, D.E. Williams, *Talanta* 38 (1991) 17.
30. M. Farrington, N. Jagota, J.M. Slater, *Analyst* 119 (1994) 233.
31. R. Sandulescu, R. Obrean, L. Roman, *Farmacia* 45 (1997) 23.
32. M.H. Pournaghi-Azar, R. Ojani, *Talanta* 44 (1997) 297.
33. J.A. Rodrigues, P. Miranda, A.A. Barros, *Port. Electrochim. Acta* 11 (1993) 41.
34. P. Cherdkiatgumachi, D.R. Grant, *Cereal Chem.* 64 (1987) 288.
35. O.W. Lau, K. Shiukwok, S.T. Chang, *J. Sci. Food Agric.* 36 (1985) 733.
36. S. Kozar, A. Beijak, J.E. Trifunovic, G. Kniewald, *Fresenius Z. Anal. Chem.* 329 (1988) 760.
37. A. Lechien, P. Valenta, H.W. Nuernberg, G.J. Patriarche, *Fresenius Z. Anal. Chem.* 311 (1982) 105.
38. S.J. Melnolasina, O. Perez Marquez, M.C. Martinez, J. Marquez, *Lebro Mem-Enuentro Nac. Electroquim.* 4 (1991) 297.
39. U. Shukla, B.B. Prasad, *Chem. Anal.* 33 (1988) 639.
40. P. Riyazuddin, S. Ali Mansoor, R. Vasanthi, *Bull. Electrochem.* 4 (1988) 295.
41. T.B. Singh, B.B. Prasad, *Chem. Anal.* 26 (1981) 541.
42. V.G. Belikov, S.V. Volokitin, *Farmatsiya* 34 (1985) 43.
43. P. Riyazuddin, J. Sankaran, *Chem. Environ. Res.* 2 (1993) 87.
44. J.J. Sun, M. Dong, H.G. Fang, H.V. Chen, *Talanta* 45 (1998) 851.

45. A.N. Strohl, D.J. Curran, *Anal. Chem.* 51 (1971) 1045.
46. C.W. Bradberry, R.N. Adams, *Anal. Chem.* 55 (1983) 2439.
47. G.M. Greenway, P. Ongomo, *Analyst* 115 (1990) 1297.
48. K. Matsumoto, T. Tsukatani, S. Higuchi, *Sens. Mater.* 7 (1995) 167.
49. L.E. Leon, *Talanta* 43 (1996) 1275.
50. M. Cheregi, A.F. Danet, *Anal. Lett.* 30 (1997) 2625.
51. O. Ffiedrich, G. Sontag, F. Pittner, *Ernachrung* 17 (1993) 605.
52. G.S. Sastry, G.G. Rao, *Talanta* 19 (1972) 212.
53. M. Tarek, M. Zaki, A.G. Abdel-Rehiem, *Mikrochim. Acta* 3 (1986) 329.
54. M. Hashmi, *Assay of Vitamins in Pharmaceutical Preparation*, Wiley/Interscience, New York, 1972, p. 286.
55. T.A.K. Nasser, A.M. Al-Rikabi, T.T. Mansoor, *Anal. Lett.* 20 (1987) 627.
56. A. Parczewski, *Acta Pol. Pharm.* 44 (1987) 442.
57. J. Montonako, S. Ikeda, N. Tanaka, *Nippon Kagaku Kaishi* 10 (1980) 1525.
58. X. Hu, Z. Leng, *Anal. Lett.* 28 (1995) 2263.
59. Y. Liu, *Huaxue Shiji* 16 (1994) 282.
60. P. Jandu, J. Weber, L. Dunsch, A.B.P. Lever, *Anal. Chem.* 68 (1996) 960.
61. J. Lie, M. Hu, R. Yu, *Sens. Actuators B* 30 (1996) 65.
62. Z. Wei, P. Guo, J. Jian, *Xibei Quinggongye Xue Yuan Xuebao* 7 (1989) 29.
63. Z. Wei, P. Guo, J. Jian, *Fenxi Huaxue* 18 (1990) 260.
64. S. Uchiyama, T. Yamaguchi, O. Hamamoto, S. Suzuki, *Bunseki Kagaku* 38 (1989) 286.
65. O. Fatibello-Filho, I. Vieira, *J. Braz. Chem. Soc.*, Vol. 11, No. 4, 412-418, 2000.
66. *The United States Pharmacopeia-The National Formulary*, USP XXXIII, Pharmacopeial Convention, Rockville, MD, p. 130, 1995.
67. H. Chem, W. Qin, Z. Zhang, *Fenxi Huaxue* 25 (1997) 1079.
68. T. Perez-Ruiz, C. Martinez-Lozano, A. Sanz, *Anal. Chim. Acta* 308 (1995) 299.
69. K. Hiromi, C. Kuwamoto, M. Ohnishi, *Anal. Biochem.* 101 (1980) 421.
70. M.I. Karayannis, *Anal. Chim. Acta* 76 (1975) 121.
71. M.I. Karayannis, D.I. Farasoglou, *Analyst* 112 (1987) 767.
72. A. Safavi, L. Fotouhi, *Talanta* 41 (1994) 1225.
73. S.M. Sultan, A.M. Abdennabi, F.E.O. Suliman, *Talanta* 41 (1994) 125.
74. Y. Ma, J. Yang, *Yaowu Fenxi Zazhi* 12 (1992) 26.
75. Y. Ma, J. Yang, *Huaxue Shejie* 31 (1990) 505.
76. T. Yamane, T. Ogawa, *Bunseki Kagaku* 36 (1987) 625.
77. H. He, Y. Shen, X. Qui, K. Ni, X. Jiang, *Zhongguo Xiyao Gongye Zazhi* 26 (1995) 70.
78. A.A. Alwarthan, *Analyst* 118 (1993) 639.
79. A.A. Ensafi, B. Rezai, *Anal. Lett.* 31 (1998) 333.
80. A. Sanz-Martinez, A. Rios, M. Valcarcel, *Analyst* 117 (1992) 1761.
81. L.E. Leon, J. Caapano, *Anal. Lett.* 26 (1993) 1741.
82. D. Liu, A. Sun, J. Liu, *Fenxi Huaxue* 23 (1995) 187.
83. K.K. Verma, A. Jain, A. Verma, A. Chaurasia, *Analyst* 116 (1991) 641.
84. A. Jain, A. Chaurasia, K.K. Verma, *Talanta* 42 (1995) 779.
85. T.F. Lin, R.F.S. Huang, T.Z. Liu, *J. Biomed. Lab. Sci.* Vol. 10, No. 1, 1998.
86. J. Peinado, F. Toribo, D. Perez-Bendito, *Analyst* 112 (1987) 775.
87. S. Feng, X. Chen, J. Fan, G. Zhang, J. Jiang, X. Wei, *Anal. Lett.* 31 (1998) 463.
88. T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, C. Sidrach, *Analyst* 122 (1995) 329.
89. Y. Fujita, I. Mori, T. Yamaguchi, M. Hoshino, Y. Shigemura, M. Shimano, *Anal. Sciences* 17 (2001) 853.
90. R. Koncki, T. Lenarczuk, S. Glab, *Analytica Chimica Acta* 379 (1999) 69-74.