ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 335–342

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Spectrophotometric determination of silicate traces in hemodialysis solutions

Maria Augusta Raggi [a,*], Cesare Sabbioni [a], Roberto Mandrioli [a], Quintilio Zini [b], Graziano Varani [c]

[a] *Dipartimento di Scienze Farmaceutiche, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Consiglio Nazionale delle Ricerche, Istituto LAMEL, Via Gobetti 101, 40129 Bologna, Italy*
[c] *Dipartimento di Chimica, University of Ferrara, Italy*

Received 12 June 1998; received in revised form 9 October 1998; accepted 25 October 1998

Abstract

Reliable methods for the analysis of silicon are of great importance, because it seems that the silicate anion can reduce aluminum bioavailability in patients undergoing dialysis. Thus, a simple and sensitive spectrophotometric method is described for the determination of silicate traces in dialysis solutions. The method is based on the reaction between silicate ions and excess ammonium molybdate reagent to give a yellow silico-molybdic complex. This complex is then reduced to the heteropoly blue compound by means of ascorbic acid. Absorbance values are measured at 830 nm, and are stable for more than 2 h. A good linearity was obtained up to 300 ng ml^{-1} of silicon concentration. The accuracy and the precision of the method were good; relative standard deviation values of 2% intraday and of 3.9% interday for six replicates on 40 ng ml^{-1} standard silicate solutions were found. Results of the analysis of some commercial hemodialysis solution samples, obtained by means of the 'standard additions' method, are provided.

Keywords: Silicon determination; Spectrophotometric analysis; Heteropoly blue complex; Dialysis solution; Standard additions

1. Introduction

Patients suffering from chronic renal failure who undergo hemodialysis can show the symptoms of anaemia, bone aches and 'dialysis dementia', a severe neurological disease. The onset of this disease has been correlated to the accumulation in central nervous system tissues of aluminum, which sometimes can be present as a contaminant in hemodialysis solutions [1–4]. In developed countries, dialysis solutions are constantly screened for the aluminium levels, but in developing countries, the aluminum contamination of dialysis solutions is still a pressing problem.

It has been recently suggested [5] that silicates can reduce aluminum bioavailability, since hydroxyaluminumsilicate complexes form under

* Corresponding author. Tel.: +39-51-259700; fax: +39-51-259734.
E-mail address: raggima@kaiser.alma.unibo.it (M.A. Raggi)

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00054-0

physiological conditions. This chemical interaction between aluminum and silicon is currently [6] considered of great interest in the biological field due to its possible role in the aluminum detoxification in patients undergoing hemodialysis.

Silicon is an essential trace element for humans, but its real biological function is still unknown. The aim of this investigation is the implementation of a simple and sensitive method for the determination of silicon levels in dialysis solutions. Silicon is not present in dialysis solutions as a normal component, but at trace levels in the form of silicic acid, $Si(OH)_4$.

Several reports on the determination of silicates in water and in biological fluids have been published; the most commonly used techniques include inductively coupled plasma-atomic emission spectroscopy [7–10], atomic absorption spectrometry [10–13], molecular absorption spectrophotometry [14], polarography [15], neutron activation analysis [16] and X-ray fluorescence spectrometry [17].

The spectrophotometric method proposed herein for silicate analysis is based on the reaction of silicic acid with ammonium molybdate and the subsequent reduction of the complex formed to heteropoly blue, which can be analyzed at $\lambda = 830$ nm. This method was found to be sensitive and reliable, and was successfully applied for the determination of trace amounts of silicon in dialysis solutions.

2. Experimental

2.1. Apparatus and materials

A Perkin Elmer (Beaconsfield, UK) model 554 double beam spectrophotometer and a Crison (Barcelona, Spain) micropH 2000 pHmeter were used. Quartz cuvettes (optical path, 1 cm) were used for the spectrophotometric analysis; after each measurement, they were washed sequentially with water, diluted hydrochloric acid, water and ultrapure water.

Ammonium molybdate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$), ferrous sulfate, sulfuric acid, 70% perchloric acid, ascorbic acid (Merck, Darmstadt, Germany), all of analytical grade, sodium metasilicate as silicon standard for atomic spectroscopy (Carlo Erba, Milan, Italy) and ultrapure water from a Millipore (Bedford, MA, USA) MilliQ apparatus were used.

Low-release polyethylene flasks and containers were used throughout the experiments because of the strength with which molybdenum blue attached to the internal surfaces of the glassware.

2.2. Solutions

The stock silicon solution (1 mg ml^{-1}, expressed as silicon) was obtained by diluting a commercial vial of sodium metasilicate to 1 l with ultrapure water; standard solutions (10 μg ml^{-1}) were prepared by diluting the stock solution with ultrapure water. The 4.8% (w/v) ammonium molybdate standard solution (38.8 mM) was prepared by dissolving 9.6 g of molybdic salt in 200 ml of ultrapure water. The 1% (w/v) ascorbic acid solution (5.68 mM) was prepared by dissolving 0.25 g of ascorbic acid in 25 ml of ultrapure water. The 35% (w/w) perchloric acid solution (6.25 M) was obtained by diluting a 70% (w/w) $HClO_4$ solution 1 + 1 with ultrapure water. The commercial dialysis solutions were provided by Sifra (Isola della Scala, Verona, Italy)

2.3. Optimal experimental conditions

In order to determine the optimal concentration of perchloric acid, and the optimal reaction conditions, sample solutions (0.80–2.00 ml of 35% perchloric acid, 1.00 ml of 1% ascorbic acid, 1.00 ml of 4.8% ammonium molybdate, 0.05 ml of 10 μg ml^{-1} silicon standard solution, ultrapure water to 25 ml) and blank solutions (prepared in the same way as the samples, except for the standard silicon solution) were thermostated at various temperatures, from 40 to 70°C. Absorbance values were measured at 5-min intervals, at $\lambda = 830$ nm.

In order to determine the optimal concentration of ammonium molybdate solution, sample solutions (1.60 ml of 35% perchloric acid, 1.00 ml of 1% ascorbic acid, 0.50–1.50 ml of 4.8% ammonium molybdate, 0.05 ml of 10 μg ml^{-1} silicon

standard solution, ultrapure water to 25 ml) and blank solutions were thermostated at 70°C for 45 min. The solutions were subsequently subjected to spectrophotometric analysis at $\lambda = 830$ nm.

In order to determine the optimal concentration of ascorbic acid solution, sample solutions (1.60 ml of 35% perchloric acid, 0.50–1.50 ml of 1% ascorbic acid, 1.00 ml of 4.8% ammonium molybdate, 0.05 ml of 10 μg ml^{-1} silicon standard solution, ultrapure water to 25 ml) and blank solutions were thermostated at 70°C for 45 min. The solutions were subsequently subjected to spectrophotometric analysis at $\lambda = 830$ nm.

2.4. Calibration curve

Into 25 ml volumetric flasks were added: 10 ml of ultrapure water, 1.6 ml of 6.25 M $HClO_4$, 1 ml of 4.8% ammonium molybdate, 1 ml of 1% ascorbic acid, different amounts of the 10 μg ml^{-1} standard silicon solution in order to obtain samples with the following concentrations: 0, 5, 10, 20, 40, 60, 100, 200 and 300 ng ml^{-1}, and ultrapure water up to volume. The calibration point were analyzed in triplicate.

The samples were thermostated for 45 min at 70°C, then the absorbance values were measured at $\lambda = 830$ nm at room temperature.

In order to speed up the procedure, 10 ml of a ready-made reagent were used. It was composed of 35% $HClO_4$:4.8% ammonium molybdate:1% ascorbic acid:ultrapure water in the ratio 1.6:1:1:6.4 (v/v/v/v). The reagent is stable at room temperature for a few days.

The standard deviation was calculated on six samples containing 40 ng ml^{-1} silicon, and using a kinetic procedure at 70°C for 45 min.

2.5. Application to dialysis solutions

Various kinds of dialysis solutions, both hemofiltration (SIF-BH 504 B and SIF-BH 499) and peritoneal dialysis (SIF-BP 466 A) solutions were analyzed. Their declared compositions are reported in Table 1.

In a 25 ml volumetric flask, to 10 ml of dialysis solution, 10 ml of ready-made reagent and ultrapure water up to volume were added. The resulting solution was thermostated at 70°C for 45 min, then subjected to spectrophotometric analysis at $\lambda = 830$ nm.

The Si levels were obtained by the 'standard addition' method. To analyze hemofiltration solutions (SIF-BH 504 B, SIF-BH 499 and 'reconstructed'), other flasks were prepared as above, but adding 50, 100 and 150 μl, respectively, of a 10 μg ml^{-1} standard silicon solution, in order to obtain a final addition of 20, 40 and 60 ng ml^{-1} silicon.

In order to apply the standard addition method to peritoneal dialysis solutions (SIF-BP466 A), other flasks were prepared as already stated, but adding 150, 200 and 250 μl, respectively, of a 10 μg ml^{-1} standard silicon solution, in order to obtain an addition of 60, 80 and 100 ng ml^{-1} of silicon.

2.6. Recovery assays

Recovery on hemofiltration solutions: to 10 ml of dialysis solution, 50 μl of a 10 μg ml^{-1} standard silicon solution were added, to obtain a final spiking of 20 ng ml^{-1}. The resulting solution was subjected to the same procedure as described in Section 2.5.

Recovery on peritoneal dialysis solutions: to 10 ml of dialysis solution, 250 μl of a 10 μg ml^{-1} standard silicon solution were added, in order to obtain a final spiking of 100 ng ml^{-1}. The resulting solution was subjected to the same procedure as described in Section 2.5.

3. Results and discussion

It is well known that molybdate (as well as tungstate) ions form polyanions in acidic medium, and that, in the presence of silicate ions, give rise to the formation of heteropolyoxyanions [18].

The reduction of the Keggin-structure anion $[SiMo_{12}O_{40}]^{4-}$ leads, in aqueous acidic solutions, to the stable heteropoly blue $[SiMo_{12}O_{40}]^{5-}$, with a peculiar intervalence band at 830 nm, due to a mixed valence species with Mo^{5+} center [19].

3.1. Preliminary studies

Preliminary assays were carried out, using a procedure previously implemented by one of the authors [20], which allowed for the determination of silicates dissolved in mineral waters. After some trials on hemodialysis solution samples, it was apparent that the results were not reliable; in particular, silicon recovery was far too high (about 130%). Subsequent attempts proved that the high ion concentration from salts contained in the dialysis solutions created strong interference, such that the method could not be directly applied to this matrix.

Drastic modifications were thus applied in order to render the procedure suitable for the determination of silicate ion levels in dialysis solutions, namely the acidic medium (perchloric acid instead of sulphuric acid), and the reducing agent (ascorbic acid instead of ferrous sulfate) were changed, thus excluding from the procedure the ferrous and sulfate ions, which were considered to be responsible for the interference.

3.2. Study of the optimal experimental conditions

The main factors that influence analysis performance are: the pH at which the silico-molybdate complex is reduced to heteropoly blue, the temperature of the reduction reaction and the reagent concentrations.

3.2.1. Perchloric acid concentration and reaction temperature

The reduction reaction on which the method is based depends strongly on the acidity of the medium. The first optimized parameter was the amount of perchloric acid in the reaction mixture. Several concentrations of acid were tried, in the 0.2–0.5 M perchloric acid range. For every concentration, a sample and a blank were analyzed at 830 nm after reaction completion. The highest

Table 1
Silicon and aluminum levels found in examined dialysis solutions

Name, type and composition	Silicon (ng ml^{-1})[a]	Aluminum (ng ml^{-1})[a]
SIF-BH 504 B (hemofiltration) NaCl, 5.73 g KCl, 0.11 g $CaCl_2 \cdot 2H_2O$, 0.22 g $MgCl_2 \cdot 6H_2O$, 0.10 g Na lactate, 4.70 g Glucose, monohydrate, 2.20 g Water for injectables to 1000 ml	25.2 ± 0.5	5.2 ± 0.1
SIF-BH 499 (hemofiltration) NaCl, 6.28 g KCl, 0.15 g $CaCl_2 \cdot 2H_2O$, 0.26 g $MgCl_2 \cdot 6H_2O$, 0.10 g Na lactate, 4.76 g Water for injectables to 1000 ml	35.4 ± 0.7	6.0 ± 0.1
SIF-BP 466 A (peritoneal dialysis) NaCl, 5.67 g $CaCl_2 \cdot 2H_2O$, 0.26 g $MgCl_2 \cdot 6H_2O$, 0.07 g Na lactate, 3.92 g Glucose, monohydrate, 16.50 g Water for injectables to 1000 ml	390 ± 8	9.5 ± 0.2
Solution reconstructed following the declared SIF-BH 504 B composition	29.8 ± 0.4	4.1 ± 0.1

[a] Each value is the mean of three determinations ± standard deviation.

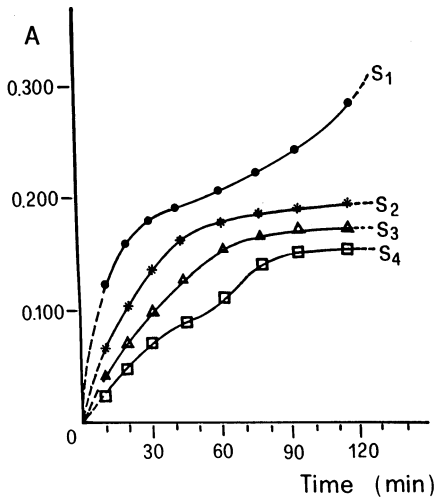

Fig. 1. Influence of temperature on reaction kinetics. Absorbances of standard silicon solutions (40 ng ml^{-1}) thermostated at various temperatures ($S_1 = 70°C$; $S_2 = 60°C$; $S_3 = 50°C$; $S_4 = 40°C$) are plotted against the time of reaction.

absorbance values are obtained in the 0.30–0.40 M range of $HClO_4$ concentration.

At the same time, the reduction kinetics of the silico-molybdate complex were studied. Results obtained by thermostating samples and blanks at temperatures between 40 and 70°C are reported in Fig. 1. At room temperature, the kinetics are very slow, and thus are not reported in the figure. At higher temperatures, however, the reaction is considerably faster, but some very high absorbance values were found after protracted exposure to high temperatures (70°C for more than 80 min). Such results can only be explained by degradation of the sample (uncomplexed molybdate probably undergoes reduction). In order to guarantee fast reaction kinetics, high temperatures as well as high concentrations of perchloric acid are thus required. A temperature of 70°C maintained for 45 min and a concentration of 0.40 M of perchloric acid were found to be the optimal parameters.

3.2.2. Concentration of ammonium molybdate reagent

The second step was the determination of the optimal concentration of the ammonium molybdate reagent.

Blanks and samples prepared as already stated, but containing various concentrations (0.78–2.33 mM) of ammonium molybdate, were thermostated at 70°C for 45 min; absorbance values at 830 nm were then determined. The results are reported in Table 2. The optimal concentration of ammonium molybdate was 1.55 mM since higher amounts caused a sharp rise in blank absorbance.

3.2.3. Amount of ascorbic acid (reducing agent)

Assays were carried out using several amounts (0.5–1.5 ml) of a 1% ascorbic acid solution. Obtained data indicate that the amount of ascorbic acid within these limits is fairly irrelevant since the absorbance difference values between samples and blanks are almost constant. This result was predictable, since the amount of ascorbic acid present is always in very large excess with respect to the silico-molybdate amount.

3.3. Method validation

A calibration curve was set up by carrying out the previously described procedure on silicate standard solutions at nine different concentrations (in triplicate), and by plotting absorbance values against silicon concentrations. Linearity was

Table 2
Determination of the optimal concentration of ammonium molybdate

Ammonium molybdate concentration (mM)	Amount of 4.8% ammonium molybdate (ml)	Blank absorbance ($\lambda = 830$ nm)	Sample absorbance ($\lambda = 830$ nm)
0.78	0.50	0.001	0.017
1.16	0.75	0.002	0.086
1.55	1.00	0.006	0.192
1.94	1.25	0.393	0.949
2.33	1.50	2.160	>2.500

found to be from 5 to 300 ng ml^{-1} silicon (regression equation: $y = 0.007 + 0.0009x$, $r = 0.999$). The limit of determination was 5 ng ml^{-1}, calculated following USP XXIII guidelines [21].

For each analysis of dialysis solution samples, the experimental calibration points were determined again in order to verify method reproducibility and reagent contamination. Under optimal conditions, reagents and water contained a total interference corresponding to a silicon concentration of about 7 ng ml^{-1} (relative standard deviation (RSD), 6.1%), a value which can be considered generally acceptable, indicating low levels of contamination.

Repeatability (or intraday precision) was evaluated on six assays performed on samples containing 40 and 10 ng ml^{-1} silicon. The obtained RSD values of 2.0 and 4.8%, respectively, indicate a good precision.

Intermediate precision was evaluated on six assays performed interday on samples containing 40 ng ml^{-1} of silicon. The RSD was 3.9%.

Regarding stability, it was found that, under these optimal conditions, the heteropoly blue complex is stable for more than 2 h at room temperature.

3.4. Determination of silicate traces in dialysis solutions

Once validated, the method was applied to the assay of silicon levels in some commercially available samples of hemofiltration solutions and of peritoneal solutions. In order to overcome the problem of sample matrix effects, the method of 'standard additions' was applied and silicon amounts in dialysis solutions were determined by means of extrapolation.

Analysis of 2.5 ml volumes of dialysis solutions (1:10 dilution) gave results which were near the quantitation limit of the method, and thus were barely reliable; subsequent analysis were thus performed using 10 ml of dialysis solution (1:2.5 dilution). The use of larger volumes of samples is not problematic, since dialysis solutions are always available in large amounts. Various volumes of 10 μg ml^{-1} standard silicon solutions were added to 10 ml aliquots of dialysis solution samples and subjected to the described procedure. Resulting absorbance values were plotted against concentrations of added silicon, and the original silicon concentrations were determined by extrapolating the least-square straight line to zero absorbance value. The assay results for a sample of solution SIF-BH504 B and the corresponding daily calibration curve are reported in Fig. 2(a) and 2(b), respectively. The difference between the x axis intercepts is 10 ng ml^{-1}, which, multiplied by 2.5 (dilution coefficient, as reported in Section 2), gives a final silicon concentration in the sample equal to 25 ng ml^{-1}.

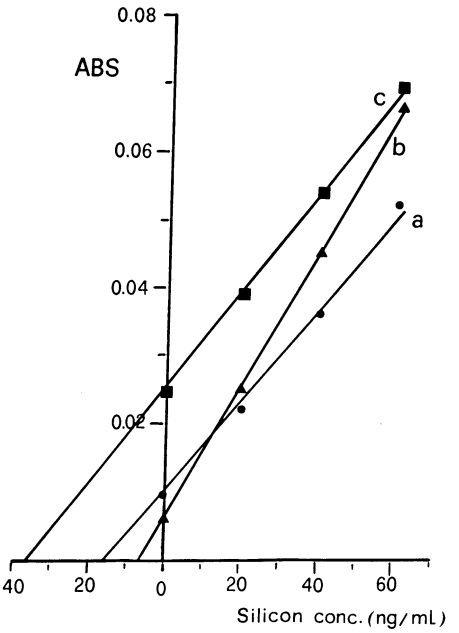

Fig. 2. (a) Determination of silicon concentration in hemodialysis solution by means of extrapolation; (b) silicon calibration curve; (c) recovery assay on the same hemodialysis solution as in (a).

The silicon levels obtained analyzing different samples of dialysis solutions are reported in Table 1, where the analysis of a 'hemodialysis solution' prepared in our laboratory is also reported, following the declared contents of a commercially available solution. Results are consistent and comparable between them (except for solution SIF-BP 466 A), as can be seen from Table 1. The high levels of silicon found in the SIF-BP 466 A solution, which is a different kind of dialysis

solution (peritoneal dialysis solution), could be abscribed to the different composition.

In order to validate the method accuracy, known amounts of standard silicon solution were added to known amount of dialysis solution samples and subjected to the described procedure. Preliminary experiments carried out by direct spiking of the samples with the standard silicon solution showed strong interference, due to silicate–matrix interactions, which give rise to flocculation or precipitation compounds. Only by adding standard silicon solutions after acidification of the sample could these interactions be eliminated.

The absorbance values obtained by means of the 'standard additions' method on a sample of hemodialysis solution (SIF-BH 504 B) spiked with 50 μl of a 10 μg ml^{-1} standard silicon solution (in order to give a final silicon addition of 20 ng ml^{-1}) are shown in Fig. 2(c). The difference between the intercepts of the zero absorbance in curves (c) and (a) gives a final value of 19.6 ng ml^{-1}, which corresponds to a silicon recovery of 98%. By analyzing all other dialysis solutions after an addition of standard silicon solution (20 ng ml^{-1} for SIF-BH 499 and 'reconstructed', 100 ng ml^{-1} for SIF-BP 466 A), recovery values of 99% for solution SIF-BH 499, of 98.2% for the 'reconstructed' solution and of 98.5% for solution SIF-BP 466 A were found. These results demonstrate the good accuracy of the method.

Moreover, Table 1 reports the values of aluminum levels found in the same samples of dialysis solutions using a recently developed spectrofluorimetric method [22]. As can be seen, the Al levels are always very low and fall within the limit (10 ng ml^{-1}) required by the European Pharmacopeia [23]. These results testify to the good manufacturing quality of the examined dialysis solutions.

4. Conclusions

The spectrophotometric method for silicic acid determination, based on the formation of the 'heteropoly blue' complex, seems to be suitable for silicate trace analysis in dialysis solutions. The method, simple and sensitive, has also the advantages of good accuracy and satisfactory precision. Investigations are currently in progress in order to verify the application of this method, after suitable modifications, to silicate anion dosage in body fluids. Clinical tests recently revealed [24] that the silicic acid influences the bioavailability of aluminium by reducing its absorption. This is of great importance, since the administration of appropriate amounts of silicate to dialysis patients could lead to a better understanding of the aluminum detoxification problem.

References

[1] M.O. Amdur, J. Doull, C.D. Klaassen. Casarett and Doull's Tossicologia, EMSI Press, Rome, Italy,1993, pp 740–742.
[2] P. Zatta, P. Zambenedetti, Le Scienze 325 (1995) 28–35.
[3] Y. Ebina, S. Okada, S. Harnazaki, O. Midorikawa, Toxicol. Appl. Pharm. 75 (1984) 211–218.
[4] J.A. Robertson, A.J. Felsenfeld, C.C. Haggood, P. Wilson, C. Clarke, F. Lloch, Kidney Int. 23 (1983) 327–335.
[5] J.D. Birchall, Chem. Br. 26 (1990) 141–144.
[6] I.H. Fahal, R. Ahmad, G.M. Bell, J.D. Birchall, N.B. Roberts, J. Anal. Atom. Spectrom. 8 (1993) 911–914.
[7] N.B. Roberts, P. Williams, Clin. Chem. 36 (1990) 1460–1465.
[8] W. Schroen, U. Mueller, Fres. J. Anal. Chem. 357 (1997) 22–26.
[9] P. Capota, G.E. Baiulescu, M. Constantin, Chem. Anal. (Warsaw) 41 (1996) 419427.
[10] K. Wrobel, E. Blanco Gonzalez, A. Sanz-Medel, J. Anal. Atom. Spectrom. 8 (1993) 915–918.
[11] J.M. Perez Parajon, A. Sanz-Medel, J. Anal. Atom. Spectrom. 9 (1994) 111–116.
[12] H.J. Gitelman, F.R. Alderman, J. Anal. Atom. Spectrom. 5 (1990) 687–689.
[13] A.J. Holden, D. Littlejohn, G.S. Fell, Anal. Proc. 29 (1992) 260–262.
[14] R.C. Saxena, H.C. Arora, Chem. Environ. Res. 3 (1994) 89–92.
[15] J. Li, H. Lai, Guilin Gongrueyuan Xueboo, 15 (1995) 276–279 [CAS 124 (1996) 607].
[16] N.I. Ward, J.A. Mason, J. Radioanal. Nucl. Chem. 113 (1987) 515–526.
[17] T. Iwamori, Y. Toriyama, T. Imaoka, X-sen Bunseiri no Shinpo 28 (1996) 187–194 [CAS 126 (1997) 910].
[18] M.T. Pope, Heteropoly and isopoly oxometalates, Springer-Verlag, Berlin, 1983 Chapter 2.

[19] J.N. Barrow, J.B. Jameson, M.T. Pope, J. Am. Chem. Soc. 107 (1985) 1771–1773.
[20] Q. Zini, P.L. Buldini, L. Morettini, Microchem. J. 32 (1985) 148–152.
[21] United States Pharmacopeia, 23rd ed. United States Pharmacopeial Convention, Rockville, MD, 1995, p. 1983.
[22] M.A. Raggi, C. Sabbioni, R. Mandrioli, Q. Zini, G. Cantelli Forti, J. Anal. Toxicol. (in press).
[23] European Pharmacopeia, 3rd ed., Council of Europe, Strasbourg, France, 1997, p. 1540.
[24] J.D. Birchall, Chem. Soc. Rev. 24 (1995) 351–357.