ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 939–945

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Chemiluminescence flow-injection analysis of captopril applying a sensitized rhodamine 6G method[1]

Z.D. Zhang, W.R.G. Baeyens*, X.R. Zhang[2], G. Van der Weken

Lab. of Drug Quality Control, Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium

Received for review 20 November 1995

Abstract

A flow-injection analysis with chemiluminescence detection is described for the determination of captopril based on the photochemical reaction with cerium(IV) in sulphuric acid medium yielding a strong chemiluminescent signal which can be sensitized by some fluorescers. The proposed procedure has a linear application range of 1×10^{-6}–2×10^{-4} M ($r = 0.997$) with a detection limit of 2×10^{-7} M, an RSD of 2.8% at 1×10^{-6} M captopril, and a sample measurement frequency of 500 h^{-1}. The method was used for the simple and rapid determination of captopril in a pharmaceutical preparation.

Keywords: Captopril; Ce(IV); Chemiluminescence; Flow-injection analysis; Rhodamine 6G

1. Introduction

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline (Fig. 1), is an orally active inhibitor of the angiotensin-converting enzyme and is widely used for the treatment of hypertensive diseases on its own or in combination with other drugs [1]. This compound can also be used to treat congestive heart failure [2].

Several methods have been reported for the quantitative determination of captopril, including titrimetry [3], spectrophotometry [4], gas chromatography (GC) [5], gas chromatography–mass spectrometry (GC–MS) [6,7], high-performance liquid chromatography (HPLC) [8,9], micro liquid chromatography [10,11], capillary zone electrophoresis (CZE) [12] and, more recently, en-

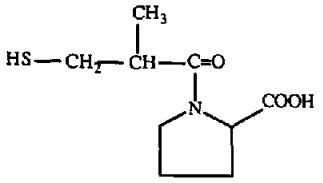

Fig. 1. Chemical structure of captopril.

* Corresponding author.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

[2] On leave from: Department of Chemistry, Shaanxi Normal University, Xian, Shaanxi 710062, China.

PII S0731-7085(95)01733-5

zyme immunoassay [13]. These methods often suffer from a variety of disadvantages: they can be sophisticated, time-consuming or require expensive instrumentation.

Some analytical procedures that exploit chemiluminescence (CL) reactions combine the advantages of speed and sensitivity with respect to spectrophotometric techniques and have been frequently used for the analysis of drugs [14–21]. When coupled with flow-injection analysis (FIA), CL-based techniques provide cheap, rapid, simple, and reproducible means of detection [22] and have been used to determine a variety of pharmaceutical products [23–25]; these characteristics are essential in drug quality control studies.

The primary goal of the present work was to develop a CL-based FIA method which is simple and fast for the routine determination of captopril in pharmaceutical preparations. The procedure proposed is based on the CL-emitting reaction between Ce(IV) and thiols in acidic medium [26,27] which could be sensitized by the use of some strong fluorophors, yielding a sensitized type of CL emission. This procedure is a good alternative for routine captopril analysis in pharmaceutical preparations.

2. Experimental

2.1. Chemicals and reagents

Captopril was purchased from Sigma Chemical Company (St. Louis, MO); ceric sulphate tetrahydrate ($Ce(SO_4)_2 \cdot 4H_2O$) and rhodamine B were obtained from U.C.B. (Leuven, Belgium); rhodamine 6G was acquired from Merck (Darmstadt, Germany). All chemicals used were of analytical-reagent grade with no further purification and deionized water was used throughout. Capoten® (25 mg Captopril tablets) was purchased from Bristol-Myers (Brussels, Belgium).

Aqueous 10 mm stock solutions of captopril were prepared by accurately weighing the pure compound into a 50 ml calibrated flask and diluting to volume with water. When not in use, the stock solution was kept at about 4°C in a dark bottle. The working solutions of lower concentrations were prepared daily by appropriate dilutions of the stock solution with water. The 2 mM Ce(IV) ion solution was prepared daily by dissolving an appropriate amount of the compound in 0.1 M H_2SO_4 medium before use because the Ce(IV) ion in solution may be reduced on air exposure (organic matter) which can easily cause errors in the experimental data. The 0.1 mM rhodamine 6G stock solution was prepared by dissolving the compound in 1 l of 0.1 M H_2SO_4. The working solutions of lower concentration were prepared by dilution of the stock solution with 0.1 M H_2SO_4 before use. The other fluorophore solutions were similarly prepared by dissolving appropriate amounts of the solid samples in 0.1 M H_2SO_4.

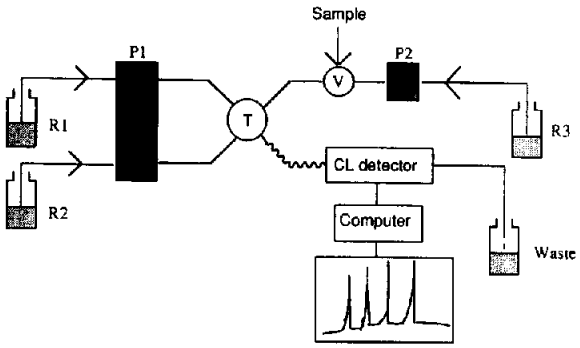

Fig. 2. Flow-injection manifold for captopril determination: R1, 2 mM Ce(IV) in 0.1 M H_2SO_4 at a flow rate of 4 ml min^{-1}; R2, 10 mM rhodamine 6G in 0.1 M H_2SO_4 at a flow rate of 4 ml min^{-1}; R3, carrier stream (water) at a flow rate of 1 ml min^{-1}; P1, peristaltic pump; P2, HPLC pump; T, mixing tee; V, injection valve (50 μl).

2.2. Apparatus

The flow-injection manifold is shown in Fig. 2. It consists of a Gilson peristaltic pump (Minipuls 2 from Gilson, two channels, variable speed), an SP 8770 isocratic pump (Spectra-Physics, CA), and a six-way 50 μl injector (Valco, The Netherlands) installed as shown. The measurements of the emitted light were made with a Bio-Orbit Oy 1250 Luminometer (Turku, Finland) and handled by an IBM-compatible computer (PC Systems) employing the luminometer software for graphical viewing of the measured values and for calcula-

Table 1
Optimized variables

Variable	Range studied	Optimized value
Cerium (IV) (mM)	0.01–10	2
Rhodamine 6G (μM)	2–25	10
Sulphuric acid (M)	0.02–0.4	0.1
Flow rate (ml min^{-1})	1–10	8
Injected sample volume (μl)	25–500	50
Coil length (cm)	2–50	2

tion. PTFE tubing (2 mm i.d.) was used throughout the manifold for carrying the CL reagents. The photoreactor was a 20 cm length of PTFE tubing (1 mm i.d.) coiled inside the measuring cell which can be turned to the detector. The inside of the cell was covered with aluminum foil so as to collect maximum reflectance of the light. Extreme precautions were taken to ensure that the cell compartment and the photomultiplier tube were light-tight.

2.3. Procedure

2.3.1. Procedure for FIA

Using the flow system schematically shown in Fig. 2, the sample solution (50 μl) was injected into a carrier solution (water) at 1 ml min^{-1}, the carrier solution was merged with the CL reagent streams (Ce(IV) in 0.1 M H_2SO_4 and fluorescer in 0.1 M H_2SO_4 at a flow rate of 4 ml min^{-1} for each) at the mixing tee, to make the CL reaction take place. The CL reagents are mixed in the coiled tube and travel about 2 cm before passing into the flow cell. The maximum light emitted by the CL reaction was detected with no wavelength discrimination.

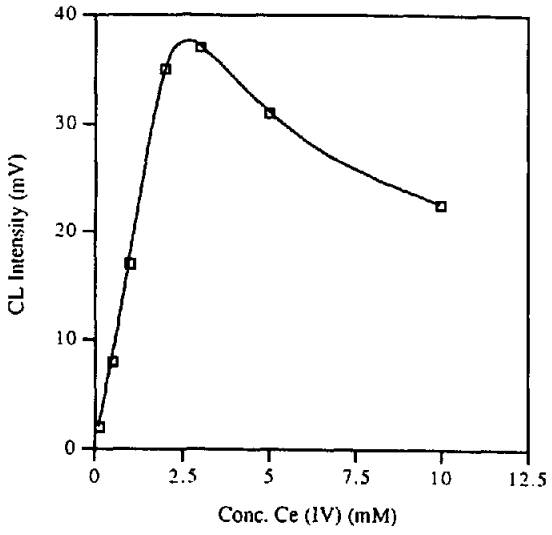

Fig. 3. Effect of Ce(IV) concentration on CL intensity of rhodamine 6G–Ce(IV)–captopril system. Ce(IV) was dissolved in 0.1 M H_2SO_4; rhodamine 6G: 10 μM in 0.1 M H_2SO_4; captopril: 10 μM.

Table 2
Effect of different fluorophores on the Ce(IV)–captopril CL emission. Ce(IV): 2 mM in 0.1 M H_2SO_4; captopril: 20 μM in water

Fluorophore	Optimized conc. (mM)	Relative CL intensity
None		10
Acridine	1	24
4,5-Dichloro-fluorescein	1	10
Eosine	0.2	17
Fluorescein	1	9
Lucigenin	1	156
Quinine	1	107
Rhodamine B	0.01	89
Rhodamine 6G	0.01	223
Riboflavin	0.1	15

2.3.2. Procedure for calibration

Using the optimum values of the variables found (2 mM Ce(IV) and 10 μM rhodamine 6G prepared in 0.1 M H_2SO_4 at a flow rate of 4 ml min^{-1} for each), a series of 10 standard solutions of captopril with different concentrations between 1 μM and 1000 μM were injected by valve (50 μl) in triplicate and a typical linear curve was obtained by plotting the CL intensity (mV) vs. captopril concentration.

2.3.3. Determination of captopril in a pharmaceutical preparation

20 tablets of the captopril preparation (label claim 25 mg captopril per tablet) were weighed to obtain the mean tablet weight. The tablets were ground to powder and an accurately weighed

portion of the powder equivalent to about 10 mg of captopril was transferred to a 200 ml volumetric flask, about 100 ml of water was added, the mixture was stirred with a magnetic stirrer for 20 min to dissolve all captopril and then diluted to volume with water. The suspension was quantitatively filtered, discarding the first portion (several millilitres) of the filtrate. 10 ml of the solution was pipetted into a 50 ml volumetric flask, diluted with water to volume, and mixed. 50 μl of the solution (containing about 50 μM captopril) was injected into the FIA manifold and used for the quantitative analysis.

3. Results and discussion

A detailed study of the variables affecting the system was performed by using the univariate method in order to define the best analytical conditions for the determination of captopril. These parameters were classified into chemical and FIA variables. The range over which these variables was studied and the optimized values are listed in Table 1.

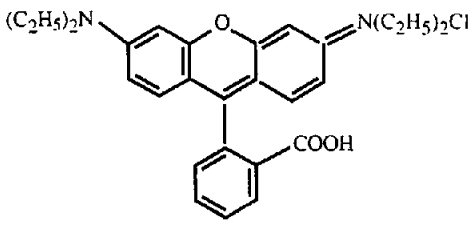

Fig. 4. Chemical structure of rhodamine B.

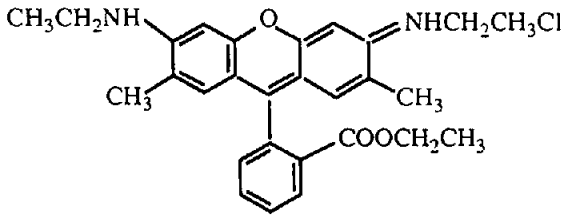

Fig. 5. Chemical structure of rhodamine 6G.

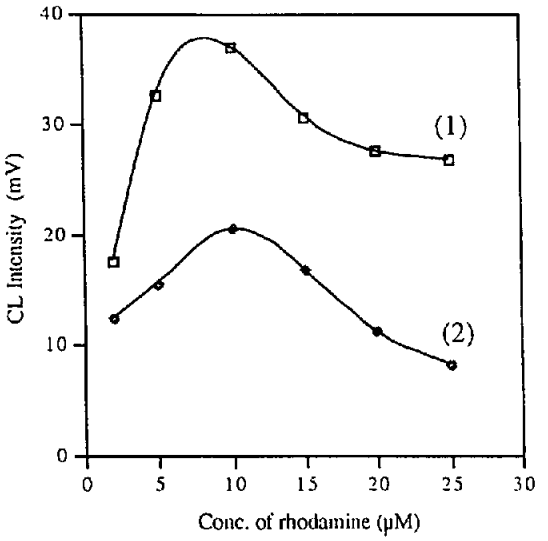

Fig. 6. Effect of rhodamine 6G (1) and rhodamine B (2) concentrations on CL emission. Ce(IV)–captopril CL emission. Ce(IV): 2 mM in 0.1 M H_2SO_4; captopril: 10 μM in water.

3.1. Optimization of the experimental CL conditions

3.1.1. Effect of Ce(IV) concentration

The effect of Ce(IV) concentration on the emission intensity in the presence of 10 μM captopril and 10 μM rhodamine B and rhodamine 6G in 0.1 M H_2SO_4 at a total flow rate of 8 ml min^{-1} is shown in Fig. 3. As the concentration of oxidant increases, the emission intensity increases up to about 2 mM of the former concentration. When the concentration of Ce(IV) exceeds 2 mM, the emission intensity decreases, because the reaction rate increases with the concentration of Ce(IV) but maximum emission occurs before the solution reaches the measuring cell. Therefore, a 2 mM Ce(IV) concentration was used for further work.

3.1.2. Effect of rhodamine 6G concentration

The CL reaction of captopril with the Ce(IV) ion can be sensitized by using some fluorescing compounds. A series of fluorescers were subjected to energy-transfer processes under various conditions and the results are shown in Table 2. Rhodamine 6G proved to be the best fluorescing compound for the envisaged CL reaction. Al-

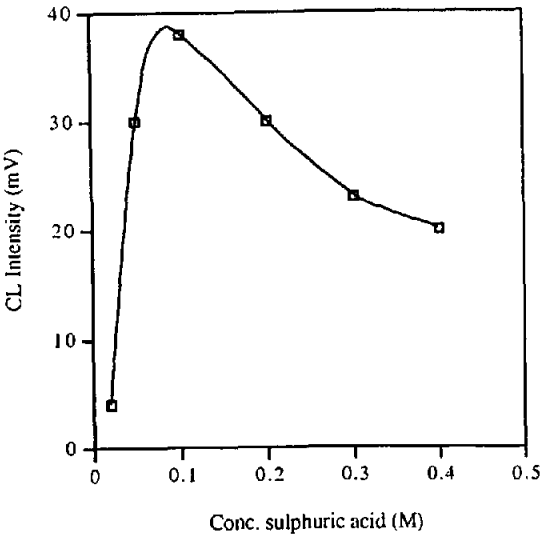

Fig. 7. Effect of sulphuric acid concentration upon rhodamine 6G-sensitized Ce(IV)–captopril CL emission. The concentrations of Ce(IV) and rhodamine 6G were 2 mM and 10 μM respectively, in 0.1 M H_2SO_4; captopril concentration was 10 μM.

though no explanation can be found so far for the exceptional behaviour of rhodamine 6G with respect to rhodamine B it is most likely that this phenomenon is due to the stereochemical alteration caused by substitution of the native rhodamine B fluorescer (Fig. 5), which produces a species that more closely matches the energy produced by the oxidative Ce(IV) action upon the analyte.

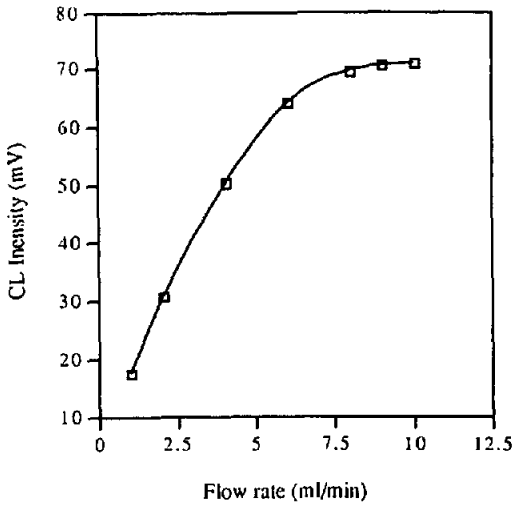

Fig. 8. Influence of total flow rate upon CL intensity. 10 μM Rhodamine 6G and 2 mM Ce(IV) in 0.1 M H_2SO_4. Captopril concentration was 20 μM.

Table 3
Recovery of 50 μM of captopril from various additives used as excipients

Additive	Additive/captopril concentration ratio (w/w)	Recovery (%) ($n = 4$)
Starch	1000	98.2
Lactose	1000	102.6
Galactose	1000	101.2
Sucrose	1000	104.8
Cellulose acetate	Saturation	97.3

The optimized concentrations of rhodamine B (Fig. 4) and 6G (Fig. 5) for the CL reaction were investigated in the range 2–25 μM. Peak heights increase with increasing rhodamine B and rhodamine 6G concentrations over the range 2–10 μM. Next, the CL signal decreases, as shown in Fig. 6. The maximum intensity of CL emission is achieved at 10 μM rhodamine 6G for the determination of captopril with this CL system.

3.1.3. Effect of sulphuric acid concentration

The effect of sulphuric acid concentration on the relative CL intensity was studied at different concentrations from 0.02–0.4 M (Fig. 7). The analytical signal increases as the concentration of sulphuric acid used to prepare the CL reagents increases up to 0.1 M; above 0.1 M the CL intensity decreases slowly. Because this CL reaction requires an acid environment, the increasing sulphuric acid concentration of the medium causes the reaction rate to also increase, resulting in the production of maximum CL emission before the solution arrives at the detection window. Hence 0.1 M sulphuric acid solution was chosen to prepare and dilute the CL reagents.

3.1.4. Effect of flow rate

The total flow rate is an important parameter in the CL reaction because the time taken to transfer the excited product into the flow cell is critical for

Table 4
Determination of captopril in a pharmaceutical preparation (Capoten®) by CL reaction with the Ce(IV) system in acidic medium

Sample	Amount (mg)		Added (mg)	Recovered (mg)	Recovery (%)
	Label	Found ± SD ($n = 10$)			
Capoten®	25.0	24.4 ± 0.72	10.0	34.63	96.3
			20.0	45.3	101.5
			30.0	53.38	94.6

maximum collection of the emitted light. The flow rate of CL reagents was varied from 1–10 ml min^{-1} and the height of the CL signal obtained for the injection of 50 μl of a solution containing 20 μM captopril was considered. The CL signal increased with increase of total flow rate up to 8 ml min^{-1} when the irradiation time was kept constant (Fig. 8). The total flow rate chosen was 8 ml min^{-1} which allows a short time interval between mixing of the reagents and measurement of the emission intensity.

3.1.5. Effect of length of the extraction coil

The effect of coil length was investigated from the minimum distance possible between mixing tee and detector up to 50 cm (1 mm i.d.) at a constant flow rate of 4 ml min^{-1}. The results showed that the CL signal increases very little with decrease of tubing length. This is because at high flow rates of the peristaltic pump, the length of the coil has no influence upon the emission profile which occurs during the time interval intersected by the observation cell. Since the tubing between the sample introduction valve and detector should have a length that allows access to the valve while protecting the detector from ambient light, a coil length of 2 cm was finally chosen.

3.1.6. Effect of injection volume

In order to check the effect of injection volume upon CL intensity, the volume of sample injection was varied from 25–500 μl of standard solution by changing the length of the sample loop in the injection valve. Above 50 μl, increasing the injection volume of the sample has little effect on CL intensity (peak height), only the width of the band increases with increasing injection volume. This is because large volumes of samples with consequent dilution effects in flowing streams influence the CL reaction and cause alterations of the CL emission profile. In order to achieve optimum application volumes, an injection volume of 50 μl proved to be suitable.

3.1.7. Interference studies

In order to assess the possible analytical applications of the described CL method, the effect of concomitant species on the determination of captopril in real samples was studied by analysing synthetic sample solutions containing 50 μM of captopril and various excess amounts of some common excipients used in the preparation of pharmaceutical formulations. A substance was considered not to interfere when the variation in captopril peak height was less than 4%. The results are shown in Table 3.

3.1.8. Validation of the method

The method was tested for linearity, precision, sensitivity and reproducibility. A series of standard solutions of captopril were injected into the manifold under the optimized conditions so as to test the linearity of the calibration graph. A linear relationship between captopril concentration (x) and CL intensity (y) was obtained over the range 50 pmol–10 nmol (50 μl per injection) with the equation: $y = 3.02x + 3.21$, with a correlation coefficient of 0.997. Under these conditions, the throughput of the method is $\approx$ 500 samples h^{-1}, the detection limit (3σ; σ is the standard deviation of the baseline) being 10 pmol. The precision of the method was studied at three concentrations under the recommended conditions. The repeatability, expressed as percent relative standard

deviation, was 2.8% obtained from 10 injections for 10 μM of captopril.

3.1.9. Application

The method was applied to the determination of captopril tablets. The recovery was checked at three concentrations of known captopril amounts in the matrix. The recoveries for three different concentrations levels (50, 100 and 150 μM) varied from 95–102%, as shown in Table 4. The method shows promise for routine control analysis of pharmaceutical preparations.

4. Conclusions

The proposed method for quantitative captopril analysis is simple and rapid (about 5 s per sample measurement only). Only small sample sizes are required (50 μl) and reproducibility is good. The linear range is satisfactory for the determination of captopril in pharmaceutical formulations. Sample manipulations are minimal and the samples can be analysed directly without any pretreatment.

The beneficial though unexpected behaviour of rhodamine 6G with respect to rhodamine B should probably be attributed to stereochemical factors producing a fluorescent species that is more efficiently excited by the energy released by the oxidative Ce(IV)–analyte reaction.

The reagents and instrumentation for the analysis are inexpensive and the method appears adequate for pharmaceutical quality control analysis. With respect to specificity, however, it is obvious that HPLC methods, though more complicated, will improve the potential of the proposed technique, and experiments in this respect are presently being considered.

References

[1] R.C. Heel, R.N. Brogdon, T.M. Speight and G.S. Avery, Drugs, 20 (1980) 409–412.
[2] J.R. Romankiewicz, R.N. Brogdon, R.C. Heel, T.M. Speight and G.S. Avery, Drugs, 25 (1983) 6–10.
[3] M.E. Mohamed, H.Y. Aboul-Enein and E.A. Gadkariem, Anal. Lett., 16 (1983) 45–55.
[4] H.T. Askal, Talanta, 38 (1991) 1115–1158.
[5] Y. Matsuki, K. Fukuhara, T. Ito, H. Ono, N. Ohara, T. Yui and T. Nambara, J. Chromatogr., 188 (1980) 177–183.
[6] A.I. Cohen, R.G. Devlin, E. Ivashkiv, P.T. Funke and T. McCormick, J. Pharm. Sci., 71 (1982) 1251–1256.
[7] O.H. Prummer, B. Jarrot and W.J. Louis, J. Chromatogr., 305 (1984) 83–94.
[8] T. Ito, Y. Matsuki, H. Kuribara and T. Nambara, J. Chromatogr., 417 (1987) 79–87.
[9] B. Lin Ling, W.R.G. Baeyens, B. del Castillo, K. Imai, P. de Moerloose and K. Stragier, J. Pharm. Biomed. Anal., 7 (1989) 1663–1670.
[10] B. Lin Ling, C. Dewaele and W.R.G. Baeyens, J. Chromatogr., 541 (1990) 189–198.
[11] B. Lin Ling, C. Dewaele and W.R.G. Baeyens, J. Chromatogr., 553 (1991) 433–439.
[12] B. Lin Ling, W.R.G. Baeyens and C. Dewaele, J. High Resolut. Chromatogr., 14 (1991) 169–173.
[13] H. Kinoshita, R. Nakamaru, S. Tanaka, Y. Tohira and M. Sawada, J. Pharm. Sci., 75 (1986) 711–713.
[14] W.R.G. Baeyens, D. De Keukeleire and K. Korkidis (Eds.), Luminescence Techniques in Chemical and Biochemical Analysis, Practical Spectroscopy Series Vol. 12, M. Dekker, New York, 1991.
[15] A.K. Campbell (Ed.), Chemiluminescence: Principles and Applications in Biology and Medicine, Ellis-Horwood, Chichester, UK, 1988.
[16] W.R.G. Baeyens, B. Lin Ling, U.A.Th. Brinkman and S.G. Schulman, J. Biolumin. Chemilumin., 4 (1989) 484–499.
[17] M. Sugiura, S. Kanda and K. Imai, Biomed. Chromatogr., 7 (1993) 149–154.
[18] S. Chen, G. Yan, M.A. Schwartz, J.H. Perrin and S.G. Schulman, J. Pharm. Sci., 80 (1991) 1017–1019.
[19] N.T. Deftereos, A.C. Calokerinos and C.E. Efstathiou, Analyst, 118 (1993) 627–633.
[20] A.B. Syropoulos and A.C. Calokerinos, Anal. Chim. Acta, 255 (1991) 403–411.
[21] I.I. Koukli, A.C. Calokerinos and T.P. Hadjiioannou, Analyst, 114 (1989) 711–714.
[22] A. Townshend, Analyst, 115 (1990) 495–500.
[23] A.A. Alwarthan and A. Townshend, Anal. Chim. Acta, 205 (1988) 261–265.
[24] P. Vinas, I. Lopez Garcia and J.A. Martinez Gil, J. Pharm. Biomed. Anal., 11 (1993) 15–20.
[25] S.A. Halvatzis, M.M. Timotheou-Potamia and A.C. Calokerinos, Analyst, 115 (1990) 1229–1234.
[26] I.I. Koukli and A.C. Calokerinos, Anal. Chim. Acta, 236 (1990) 463–368.
[27] X.R. Zhang, W.R.G. Baeyens, G. Van der Weken, A.C. Calokerinos and K. Nakashima, Anal. Chim. Acta, 303 (1995) 121–125.