Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 689–695
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A single spectroscopic flow-through sensing device for determination of ciprofloxacin

Mª Isabel Pascual-Reguera*, Gertrudis Pérez Parras, Antonio Molina Díaz

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, E-23071 Jaén, Spain

Received 4 February 2003; received in revised form 25 July 2003; accepted 8 August 2003

Available online 10 May 2004

Abstract

A simple flow injection UV spectrophotometric sensing device was developed for the determination of ciprofloxacin. The method is based on its transient retention and concentration on Sephadex SP C-25 cation-exchange gel beads packed in the flow cell and the continuous monitoring of its native absorbance on the solid phase at 277 nm. The procedure is carried out without any derivatisation. Formic acid/NaOH 1.75 M at pH 2.2 is used as carrier solution in a simple monochannel FIA manifold. When the analytical signal reached the maximum value, ciprofloxacin was eluted from the solid support by the carrier solution itself. The response of the sensor was linear in the concentration range 0.5–10 $\mu g\,ml^{-1}$ with an RSD (%) of 0.79, a detection limit (3σ criterion) of 0.035 $\mu g\,ml^{-1}$ and a sampling rate of 16 h^{-1}. Application to the analysis of pharmaceutical samples testifies the utility of this sensor.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Solid phase spectrophotometry; Flow injection analysis; Ciprofloxacin; Flow-through sensor

1. Introduction

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) (Fig. 1) belongs to the quinolones which are synthetic antibiotics, chemically related to nalidixic acid. These drugs form a group of antimicrobial agents with different chemical structures and spectra of activity.

Almost all of the recent clinically useful quinolones bear a fluorine atom in the C-6 position of the quinolone and these antibacterial agents are called fluoroquinolones.

Ciprofloxacin (belonging to the second-generation fluoroquinolone) is the most potent fluoroquinolone against Gram-positive and Gram-negative bacteria through inhibition of their NAD gyrase, a critical enzyme to bacterial chromosome replication. It is used in a wide range of gastrointestinal urinary and respiratory tract as well as ocular and skin infections and it is particularly active against pseudomonas aeruginosa.

Therefore it is necessary to arrange sensitive and fast methods for determination of this antibacterial agent. Numerous methods have been reported for the determination of ciprofloxacin using techniques such as spectrophotometry (indirectly [1,2]; by means of a charge transfer complex formation [3]; by using its Cu(II) complex and derivative spectrophotometry [4], direct UV spectrophotometry [5] or derivative

* Corresponding author. Fax: +34-953-012141.
E-mail address: ipascual@ujaen.es (M.I. Pascual-Reguera).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.03.002

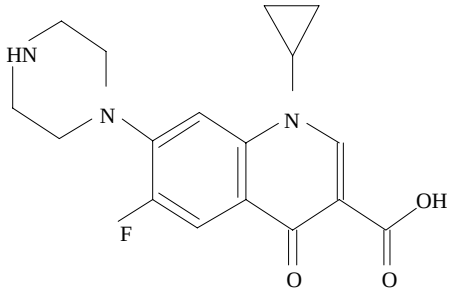

Fig. 1. Structure of ciprofloxacin.

UV spectrophotometry [6]); high performance liquid chromatography with UV detection [7]; capillary electrophoresis [8–10] and immunoassay [11]. Automatic methods based on flow injection analysis (FIA) have also been proposed using various spectroscopic detection techniques: spectrophotometry [12], luminescence (spectrofluorimetry [13,14], phosphorimetry [15] and chemiluminescence [16]). A sequential injection analysis (SIA) procedure has also been described [17].

The aim of this work was to develop a single, fast and inexpensive analytical method for determination of ciprofloxacin based on SPS–FIA systems. Solid phase spectrophotometry (SPS) is a methodology appeared in 1976 [18] which has been applied to a variety of inorganic [19] and organic analytes [20]. The methods based on this technique offer a very high sensitivity and cheap instrumentation but they may be tedious and time-consuming. The SPS–FIA is a new technique based on the retention of the analyte (or any of the component of a chemical reaction) on an active solid support placed in an appropriate flow cell by using a non-destructive optical detector (photometric or fluorimetric). The preconcentration of the specie of interest is not prior to the measurement but simultaneous. Thus, these systems integrate simultaneously in time and space several analytical processes: retention/separation, preconcentration and detection. Theses approaches are called flow-through chemical sensors [21].

The retained species are eluted after reaching the detection zone and developing the analytical signal, in order to regenerate the solid sorbent. The SPS–FIA integration combines the advantages of the continuous flow system (rapidity, automation) with those of SPS. Versatility, economy, lower consumption of reagent and, in some cases, absence of derivative reagent are the advantages of this new approach.

Several of these SPS–FIA combinations have been developed for inorganic [22–24] and organic species [25] by using derivative reagents.

SPS–FIA system based on the measurement of the intrinsic absorbance of the analyte in the UV region for pharmaceutical analyses have been previously published [26–28].

In this paper we propose a spectrophotometric continuous flow sensor for determination of ciprofloxacin based on its the transitory retention on a cationic exchanger gel in the detection zone of a UV spectroscopic detector after the injection of the sample solution in an appropriate carrier stream. When the sample plug reaches the solid phase located in the flow cell, ciprofloxacin is retained on it and the signal corresponding to its intrinsic absorbance is monitored at 277 nm. After each measurement, the sensing solid microzone is regenerated by means of the carrier stream solution itself and hence rendered ready for subsequent analysis. The sensor has been successfully applied to the determination of ciprofloxacin in several pharmaceuticals preparations.

2. Experimental

2.1. Reagents

All chemicals were of analytical-reagent grade. Ciprofloxacin (Acofarma, Madrid, Spain) stock solution 100 $\mu g\,ml^{-1}$ in 0.05 M HCl aqueous solution. Working solutions were prepared daily by suitable dilution with 0.05 M HCl.

Sephadex SP-C25 (Aldrich, Madrid, Spain) ion-exchanger without previous pre-treatment was used as solid support placed inside a 1 mm Hellma 138-QS quartz flow-through cell (50 μl inner volume) with glass wool in the outlet to prevent gel beads movement.

The carrier/self-eluting solution used consisted of 1.75 M formic acid/NaOH at pH = 2.2.

2.2. Dosage forms of ciprofloxacin

(1) Ringoran tablets (Vita Laboratory): Ciprofloxacin hydrochloride 500 mg; (2) Estecina tablets

(Normon Laboratory, Spain): Ciprofloxacin hydrochloride 500 mg with excipients; (3) Oftacifox colirium (Alcon Laboratory, Spain): Ciprofloxacin 3 mg ml^{-1} with excipients; (4) Belmacina tablets (Cepa Laboratory, Spain): Ciprofloxacin hydrochloride 250 mg with excipients; (5) Catex tablets (Cantabria Laboratory, Spain): Ciprofloxacin hydrochloride 250 mg with excipients; (6) Baycip capsules (Bayer Laboratory): Ciprofloxacin hydrochloride 500 mg with excipients.

2.3. Apparatus and flow diagram

The FI system consisted of a GILSON MINIPULS 3 (Villiers-Le-Bel, France) peristaltic pump, one rotary valve (RHEODYNE 50), a 1 mm Hellma 138-QS quartz flow-through cell (50 μl inner volume) with the ion exchanger gel and PTFE connecting tube (0.8 mm inner diameter).

All spectral measurements and real-time data acquisition of flow injection peaks were made with a single beam microprocessor controlled UV-Vis Unicam 8625 spectrophotometer, from Unicam Analytical Systems, connected to a 386 IBM personal computer by a serial port and fitted with the 8625 Rate software package (from Unicam) for data acquisition and processing. Spectra were registered at a rate of 250 nm min^{-1}. Absorbance measurements were carried out as explained in a previous paper [29].

The pH measurements were made with a Crison Model 2002 (Barcelona, Spain) pH-meter fitted with a glass saturated calomel electrode assembly and a temperature probe.

The single channel manifold used is shown in Fig. 2.

2.4. Procedure for calibration

The sample solution (200 or 600 μl) containing 1–30 or 0.25–12 μg ml^{-1} of ciprofloxacin was inserted into the carrier stream (HCOOH/NaOH, total concentration 1.75 M) at a flow-rate of 1.23 ml min^{-1}. The analyte was transported through the flow cell where it was sorbed on the cationic Sephadex SP-C25 resin. The increase in absorbance was continuously monitored at a wavelength of 277 nm in the personal computer and sent to the printer. Two different calibration lines were constructed in the concentration ranges and sample volumes indicated above. After

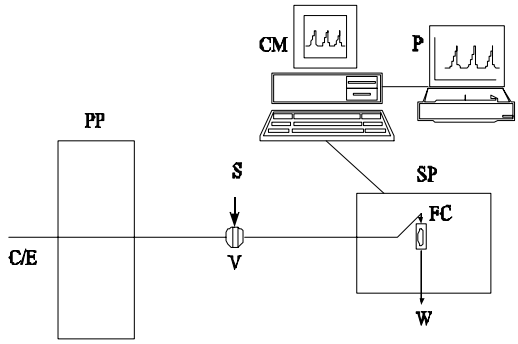

Fig. 2. Manifold. PP: peristaltic pump. C/E: carrier/eluting solution flowing at 1.23 ml min^{-1}; V: injection valve with appropriate sample volume loop; S: sample; FC: flow cell; W: waste; SP: Spectrophotometric detector tuned at 277 nm; CM: computer; P: printer.

reaching the maximum signal, ciprofloxacin was desorbed from the flow-through cell by the carrier itself, therefore regenerating the active ion-exchanger gel microzone, and allowing the absorbance value to return to the baseline (Fig. 3).

2.5. Treatment of samples

Six tablets were weighed and crushed to powder in agate mortar. An amount of the powder equivalent to one tablet was accurately weighed, dissolved in HCl 0.05 M, filtered through a 0.45 μm pore size Millipore membrane filter and the filtrate and washing

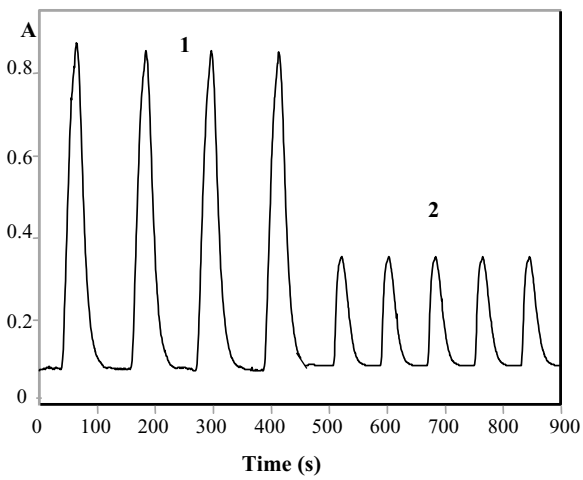

Fig. 3. Fiagram obtained for a sample volume of 600 μl. (1) Flow cell with solid support; (2) flow cell without solid support.

were made up to an appropriate volume with distilled water to obtain a ciprofloxacin concentration of 0.25–10 μl ml^{-1}. Octacifox colirium was appropriately diluted with 0.05 M HCl solution.

3. Results and discussion

3.1. Solid support selection

Several Sephadex cationic ion-exchangers (CM-C25 and SP-C25), Sephadex anionic ion-exchangers (QAE-A25 and DEAE-A25) and filtration gels different bead sized (G 25 and G 100 Sephadex) were tested as solid supports placed in the cell. Six hundred microlitres of a 5 mg l^{-1} ciprofloxacin solution was injected into a carrier stream of 1.75 M pH 2.2 formic acid/NaOH buffer solution. Solid phases with matrices containing aromatics rings (such as Dowex resins) were disregarded due to their incompatibility with detection in UV region; they showed such a strong absorption, which disabled light measurement in this range. The highest absorbance values were found with Sephadex SP-C 25 and were used throughout this work.

3.2. Spectral characteristics

The spectrum of ciprofloxacin (1.29×10^{-5} M) from an injection of 600 μl in the single channel manifold at a pH value of 2.2 was obtained in a Hellma 138 QS cell (1 mm optical path length without solid support) by stopped flow. It showed a maximum at 270 nm with a net absorbance value of 0.25. When the same volume of the same solution was injected with the solid support in the cell, a slight bathochromic shift of the maximum (277 nm), as well as a noticeable increase of the net absorbance value (0.8) were observed (Fig. 4).

This increasing in the absorbance signal is a consequence of the preconcentration of the analyte on the solid support in the detection zone itself. This is a general result in this kind of approach. Fig. 4 shows an FIA curve obtained with and without the sorbent. Together with this high increase of sensitivity the solid support states a higher selectivity owing to those species which can not be retained on the sensing zone are excluded from the detection area.

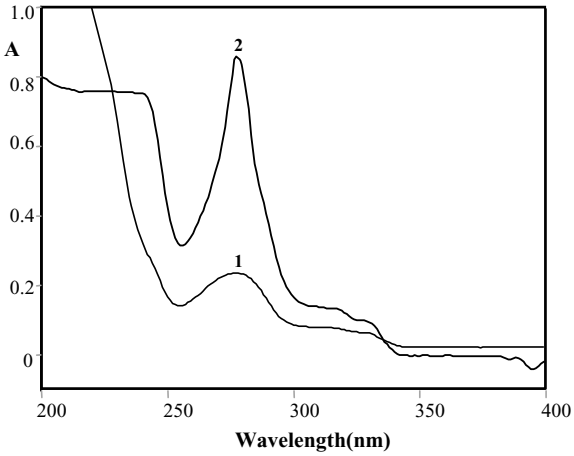

Fig. 4. Absorption spectra of ciprofloxacin in solution flow (without solid support) (1) and sorbed on Sephadex SPC C 25 (2). [ciprofloxacin] = 1.29×0^{-5} M; 600 μl of injected sample; 1 mm optical path length.

3.3. Study of experimental variables

3.3.1. Amount of solid support in the cell

The beads, as a slurry suspension in water, were introduced in the flow cell with the aid of a syringe. After conditioning them by passing the carrier solution for a few minutes, the solid support was ready for use. The level of solid support in the flow cell must be taken into consideration. This level has to be sufficiently high to allow the light beam to pass through only the gel beads (and not the solution) but if it is too high, the analyte is retained on a non-irradiate area of the solid support (above the light beam) [24]. This level depends on the geometry of the light beam, the flow cell and the sample compartment. In this case the optimum level of solid support was 15 mm from the bottom of the cell. This level of solid support in the cell is easy to reproduce because it is just the height of the measurement cell and hence the packing reproducibility is satisfactory.

3.3.2. Chemical variables

The influence of the carrier solution pH on the retention–elution of ciprofloxacin was studied in the pH region 1–5, and using 600 μl of a 2.6×10^{-5} M ciprofloxacin solution. At slightly basic pH values, the analyte was unstable. The optimum pH value was 2.2.

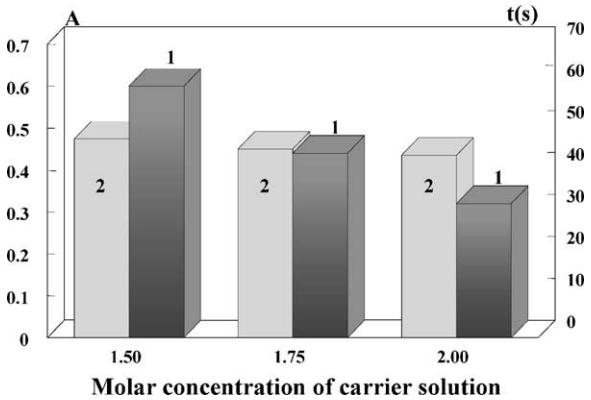

Fig. 5. Influence of eluting time (1) and concentration of carrier solution buffer (2) on the signal.

Citric acid/sodium hydroxide, potassium hydrogen phthalate acid/hydrochloride acid and formic acid/sodium hydroxide were tested at pH 2.2. Formic acid/NaOH gave the highest signal. Various concentrations of carrier/eluting solution of formic acid/NaOH were tested from 1.5 to 2.0 M by injecting 600 μl of a ciprofloxacin concentration of 1.29×10^{-5} M. As the concentration increased, the sampling frequency increased as a result of the peak width decreasing, also decreasing the peak height. This is due to the eluting effect of the anions from the carrier/eluting solution by competition for the active sites of the ion exchange gel. So, for an 1.5 M concentration, the eluting time was 60 s; and for a 2.0 M concentration, 32 s, but the peak height was lower than that using an 1.5 M concentration (84%) (See Fig. 5). Therefore, a 1.75 M concentration was chosen, which gave a 52% peak height with respect to use 1.5 M and an eluting time of 44 s, with a complete baseline restoration.

3.3.3. FIA variables

As expected, an increase in the flow rate produced a decrease in peak heights due to the lower contact time between the analyte and the beads. Moreover, high flow rates caused an increase in pressure drop because of the compaction of the solid support. On the other hand, the lower the flow rate, the lower the sampling frequency. Thus, a flow rate of 1.23 ml min^{-1} was selected as a value of compromise.

In continuous flow through solid phase spectrophotometric systems, the use of a higher injection volume results in an increase in sensitivity, as a consequence of greater amount of analyte retained in the same amount of active solid support. The effect is similar to that in SPS batch methodology [30], and it allows working with various ranges of analyte concentration, by simple changing the sample volume injected, and/or reducing matrix effects by appropriate dilution of the samples. In the system under study the influence of sample volume was studied by inserting loops of different volume between 0.2 and 2.0 ml. Much higher sensitivity (six times) was obtained by employing larger volume of sample solution, but more time was required for each determination and the sample throughput decreased. So, the selection of sample volume should reflect a consideration of both sensitivity and analysis speed. We have chosen 0.6 ml of sample volume to calibrate the sensor. Nevertheless, it would be possible to select the most appropriate volume of sample taking into account the sample concentration that were going to be analysed.

3.3.4. Analytical figures of merit

Using above conditions, the calibration graph was established for 200 and 600 μl sample volume with standard solutions of ciprofloxacin. The figures of merit of the method proposed for the two calibration volumes are summarised in Table 1.

Ten blank solutions were measured to calculate the detection and quantification limits. The calculations were performed using the 3σ [31] and 10σ [32] recommendations, respectively.

At least, 300 sequential determinations can be carried out without needing to change the solid support.

Table 1
Analytical figures of merit

Parameter	Volume of sample injected	
	200 μl	600 μl
Intercept	−0.012	0.024
Slope (ml μg^{-1})	0.029	0.079
Linear dynamic range (μg ml^{-1})	1–35	0.25–10
Correlation coefficient	0.998	0.997
Detection limit (μg ml^{-1})	0.125	0.035
Quantification limit (μg ml^{-1})	0.420	0.120
RSD (%) ($n = 10$)	2.78	0.79
Sampling frequency (h^{-1})	18	16

Table 2
Determination of ciprofloxacin in pharmaceuticals and recovery study

Pharmaceutical	Amount added	Amount found ± σ	Recovery (%)
Ringorán[a]	–	495 ± 10	–
	250	750 ± 10	102
	500	990 ± 10	99
	750	1260 ± 10	102
	1000	1490 ± 20	99.5
Estecina[a]	–	490 ± 6	–
	250	743 ± 3	101.2
	500	980 ± 8	98
	750	1250 ± 10	101.3
	1000	1480 ± 10	99
Belmacina[a]	–	255 ± 8	–
	125	390 ± 2	100.8
	250	500 ± 2	98
	375	640 ± 5	102.7
Catex[a]	–	253 ± 8	–
	125	395 ± 5	97.6
	250	505 ± 3	100.8
	375	620 ± 5	97.9
Baycip[a]	–	490 ± 10	–
	250	750 ± 6	104
	500	990 ± 8	100
	750	1240 ± 5	100
	1000	1510 ± 15	102
Oftacilox[b]	–	3.0 ± 0.1	–
	1.5	4.4 ± 0.1	100
	3.0	5.9 ± 0.1	96.7
	4.5	7.4 ± 0.2	97.8

[a] Amount expressed as mg per unit ± standard deviation.
[b] Amount expressed as mg ml^{-1} ± standard deviation.

3.4. Influence of foreign species

A study of interferences evaluated the potential effect of foreign species in the determination of ciprofloxacin. A foreign species was considered not to interfere if it produced an error of ∼5% in the determination of the analyte. The species assayed were those commonly found along with ciprofloxacin as excipients and additives in pharmaceuticals preparations, namely, talc, glucose, lactose, sucrose, saccharin, starch, citrate, dextrose and ethanol. None of them interfere in the determination at the highest level assayed (interference/ciprofloxacin ratio = 25).

3.5. Analytical applications

Six commercially available dosage forms were analysed by using the proposed sensor and 600 μl of sample. Results are summarised in Table 2. As can be seen, there is a good agreement between the amounts found and those claimed by the manufacturer. In order to check the accuracy of the proposed sensor, recovery studies were performed. Known amounts of ciprofloxacin standard were added in triplicate to pharmaceuticals assayed. Very satisfactory recovery values (ranging from 96.7 to 104%) were found.

4. Conclusions

A procedure for determination of ciprofloxacin is proposed by implementing FIA with direct solid phase UV detection. The continuous flow solid phase UV spectrophotometric system is fast, simple and shows high sensitivity and low cost per analysis. The intrinsic absorbance of ciprofloxacin is the analytical signal, so no derivatization reaction is required. Neither sophisticate nor expensive instrumentation is needed. The sensor has proved to be suitable for determination of the fluoroquinolone ciprofloxacin in pharmaceuticals with suitable accuracy and precision as a routine method. Finally the increase of sensitivity introduced by the use of a solid sensing support, combined with that originated by the use of higher sample volume could also be exploited by studying the possibility of applying it to the determination of ciprofloxacin in biological fluids.

Acknowledgements

The authors are grateful to the Ministerio de Educación y Cultura, Dirección General de Enseñanza Superior (DGES) (Project No. PB98-0301) for financial support.

References

[1] A.F. el Walily, S.F. Belal, R.S. Barry, J. Pharm. Biomed. Anal. 14 (1996) 561–569.
[2] B.S. Nagaralli, J. Seetharamappa, M.B. Melwanki, J. Pharm. Biomed. Anal. 29 (2002) 859–864.

[3] S. Mostafa, M. El-Sadek, E.A. Alla, J. Pharm. Biomed. Anal. 27 (2002) 133–142.
[4] M. Rizk, F. Belal, F. Ibrahim, S. Ahmed, Z.A. Sheribah, J. AOAC Int. 84 (2001) 368–375.
[5] The United States Pharmacopoeia, XXVI Revision, 2003.
[6] H. Hopkala, D. Kowalczuk, Acta Poloniae Pharm. 57 (2000) 3–13.
[7] M. Kamberi, N. Hajime, P. Kamberi, N. Uemura, K. Nakamura, S. Nakano, Therp. Drug Monit. 21 (1999) 335–340.
[8] D. Barrón, E. Jiménez-Lozano, J. Cano, J. Barbosa, J. Chromatogr. B 759 (2001) 73–79.
[9] M. Hernández, C. Aguilar, F. Borrull, M. Calull, J. Chromotogr. B: Anal. Technol. Biomed. Life Sci. 772 (2002) 163–172.
[10] C. Fierens, S. Hillaert, W. Van den Bossche, J. Pharm. Biomed. Anal. 22 (2000) 763–772.
[11] G.G. Snitkoff, D.W. Grabe, R. Holt, G.R. Bailie, J. Immunoassay 19 (1998) 227–238.
[12] S.M. Sultan, F.O. Suliman, Analyst 117 (1992) 1523–1526.
[13] A. Zotou, N. Miltiadou, J. Pharm. Biomed. Anal. 28 (2002) 559–568.
[14] M.A. García, C. Solans, J.J. Aramayona, S. Rueda, M.A. Bregante, A. De Jong, Biomed. Chromatogr. B 13 (1999) 350–353.
[15] R. Cuenca Trujillo, M.J. Ayora Cañada, A. Molina Díaz, J. AOAC Int. 85 (6) (2002) 1268–1272.
[16] F.A. Aly, S.A. Al-Tamimi, A.A. Alwarthan, Talanta 53 (2001) 885–893.
[17] F.O. Suliman, S.M. Sultan, Talanta 43 (1996) 559–568.
[18] K. Yoshimura, H. Waki, S. OACI, Talanta 23 (1976) 449.
[19] P. Ortega-Barrales, A. Molina-Díaz, M.I. Pascual-Reguera, L.F. Capitán-Vallvey, Anal. Chim. Acta 353 (1997) 115–122.
[20] A. Ruiz Medina, M.L. Fernández de Córdova, A. Molina Díaz, J. Pharm. Biomed. Anal. 29 (1999) 247–254.
[21] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 173 (1985) 3–21.
[22] K. Yoshimura, Analyst 113 (1988) 471.
[23] L.F. Capitán-Vallvey, M.C. Valencia, G. Mirón, Anal. Chim. Acta 289 (1994) 365–370.
[24] M.J. Ayora Cañada, M.I. Pascual Reguera, A. Molina Díaz, Anal. Chim. Acta 375 (1998) 71–80.
[25] D. Chen, M.D. Luque de Castro, M. Valcárcel, Anal. Chim. Acta 261 (1992) 269–274.
[26] M.J. Ayora Cañada, M.I. Pascual Reguera, A. Molina Díaz, Int. J. Pharm. 202 (2000) 113–120.
[27] M.J. Ayora Cañada, M.I. Pascual Reguera, A. Ruiz Medina, M.L. Fernández de Córdova, A. Molina Díaz, J. Pharm. Biomed. Anal. 22 (2000) 59–66.
[28] M.I. Pascual Reguera, M.J. Ayora Cañada, M.S. Castro Ruiz, Eur. J. Pharm. Sci. 15 (2002) 179–183.
[29] A. Molina Díaz, A. Ruiz Medina, M.L. Fernández de Córdova, J. Pharm. Biomed. Anal. 28 (2002) 399–419.
[30] M.L. Fernández de Córdova, A. Molina Díaz, M.I. Pascual Reguera, L.F. Capitán Vallvey, Talanta 42 (1995) 1057.
[31] IUPAC, Nomenclature, symbols, units and their usage in spectrochemical analysis—II, Spectrochim. Acta B 33 (1978) 242–245.
[32] ACS Committee on Environmental Improvement. Guidelines for data acquisition and data quality evaluation in environmental chemistry, Anal. Chem. 52 (1980) 2242.