ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 23–29

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

# Differential-pulse adsorptive stripping voltammetric determination of the antibacterial lomefloxacin

José Luis Vílchez, Lilia Araujo [1], Avismelsi Prieto [2], Alberto Navalón *

*Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/. Fuentenueva s/n, E-18071 Granada, Spain*

Received 27 October 2000; received in revised form 27 December 2000; accepted 2 January 2001

**Abstract**

A differential-pulse adsorptive stripping voltammetric method for the determination of trace amounts of the antibacterial lomefloxacin is proposed. By using an accumulation potential of $-0.30$ V and a 2 min accumulation time, the linear concentration range of application was 1.0–10.0 ng $ml^{-1}$ of lomefloxacin, with a relative standard deviation of 3.8% (for a level of 5.0 ng $ml^{-1}$) and a detection limit of 0.3 ng $ml^{-1}$. The method was applied to determination of lomefloxacin in human urine and serum samples. It was validated using HPLC as a reference method. Recovery levels of the method reached 100% in all cases. 

*Keywords:* Differential-pulse voltammetry; Adsorptive stripping; Antibacterials; Lomefloxacin

## 1. Introduction

Lomefloxacin (LFLX) [1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylix acid] (Fig. 1) is one of the synthetic antibacterial fluoroquinolone agents of the third generation, which exhibits high activity against a broad spectrum of Gram-negative and Gram-positive bacteria. This synthetic fluoroquinolone derivative is used for the control of the urinary tract and respiratory infections [1].

LFLX has been found in body tissues, blood, serum and urine a few hours after oral administration. After an oral dose of 400 mg of LFLX, the average concentration of LFLX in the urine and serum samples within 24 h were in the ranges of 332–41 and 4–0.2 μg $ml^{-1}$ [2], respectively. The excretion and metabolism of LFLX has been investigated [1]. Urine seems to be the major route of excretion (70–80%). LFLX was not extensively metabolised and only 6.1% was excreted changed. Glucuronidation was the major metabolic pathway involved in the elimination.

* Corresponding author. Tel.: +34-958-243326; fax: +34-958-243328.

*E-mail address:* anavalon@ugr.es (A. Navalón).

[1] On leave from the Faculty of Engineering, University of Zulia, Maracaibo, Venezuela.

[2] On leave from the Faculty of Agronomy, University of Zulia, Maracaibo, Venezuela.

0731-7085/01/$ - see front matter 
PII: S0731-7085(01)00391-0

The widespread use of this compound and the need for clinical and pharmacological study require fast and sensitive analytical techniques to determine the presence of the drug in several biological fluids. Up to now the most common techniques for the determination of the drug in commercial formulations and biological fluids have been based on HPLC with UV detection [3] or with fluorimetric detection [4] methods, but capillary zone electrophoresis [5], micellar electrokinetic capillary chromatographic [6], spectrophotometric [7] and photochemical-fluorimetric [2] methods, have also been used.

Stripping voltammetry (SV) comprises a variety of electrochemical approaches, having a step of preconcentration onto the electrode surface prior to the voltammetric measurement. For the trace analysis of inorganic and organic compounds that cannot be accumulated by electrolysis, the stripping method proposed has been the adsorptive stripping voltammetry (AdSV). In AdSV, the analyte is adsorbed on the working electrode by means of a non-electrolytic process prior to the voltammetric scan [8]. The high sensitivity of adsorptive stripping methods makes it possible to work with very diluted samples with a corresponding decrease in possible interferences in the analysis.

In this paper, a differential-pulse adsorptive stripping voltammetric method for the determination of lomefloxacin is proposed. The method has been applied to the determination of LFLX in human urine and serum samples.

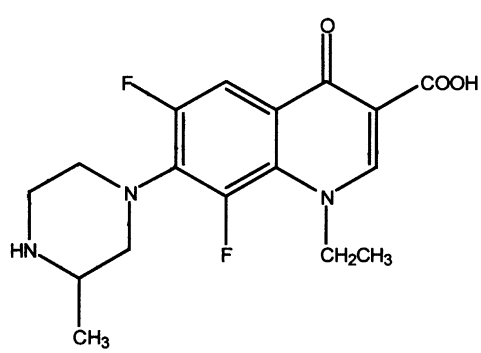


Fig. 1. Structure of lomefloxacin.

## 2. Experimental

### 2.1. Reagents

Stock solution (1.0 mg $ml^{-1}$) of lomefloxacin was prepared by exact weighing of the lomefloxacin hydrochloride (Sigma) and dissolution in 0.04 M acetate buffer solution of pH 4.0 (supporting electrolyte). The solution was stable for at least 4 weeks, if stored in the dark at 4°C. Working solutions were prepared daily by appropriate dilutions with supporting electrolyte.

The 0.04 M acetate buffer solution of pH 4.0 used as supporting electrolyte was prepared from sodium acetate (Merck) and acetic acid (Merck). All reagents were of the analytical-reagent grade unless stated otherwise. Aqueous solutions were prepared in water purified with a Milli-Q plus system (Millipore).

### 2.2. Apparatus and software

Adsorptive and voltammetric experiments were performed using an Autolab (Eco Chemie BV) PGSTST10 potentiostat/galvanostat in conjunction with a Metrohm 663 V stand. A three-electrode system was composed of a static mercury dropping electrode (SMDE), Ag/AgCl reference electrode and a glassy carbon auxiliary electrode.

PGSTAT10 potentiostat/galvanostat was interfaced with an ADL Pentium MMX 200 microcomputer supplied with General Purpose Electrochemical System (GPES) software (Eco Chemie BV) for data acquisition and its subsequent analysis.

All pH measurements were made with an Ingold combined glass-saturated calomel electrode using an earlier calibrated Crison 2000 digital pH-meter.

Statgraphics [9] and Alamin [10] software packages were used for the statistical treatment of the data and regression analysis (linear model) and statistical treatment of data.

### 2.3. Sample treatment

Urine samples were obtained from healthy male volunteers and the serum samples were supplied

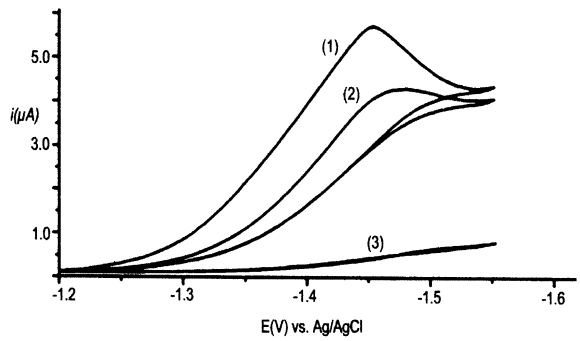


Fig. 2. Cyclic voltammograms of lomefloxacin: (1) accumulation time of 30 s. (2) without accumulation time. (3) Cyclic voltamograms of serum (100 μl) with and without 30 s accumulation time. [LFLX] = 400 ng $ml^{-1}$; pH 4.0 (acetate buffer); accumulation potential = − 0.3 V.

by the Virgen de las Nieves Hospital (Granada). Urine samples were centrifuged for 10 min at 3800 rpm and filtered through a Minisart-plus syringe filter (0.2 μm pore size, Supelco). Serum samples were filtered through a Centricon 3 centrifugal filter (Amicon) at 7000 rpm.

The filtrates were collected in glass containers that had been carefully cleaned with nitric acid and washed with deionised water and stored at 4°C until analysis was performed with the minimum possible delay. Aliquots of these filtrates were taken and treated as described in Section 2.4.

### 2.4. Analytical procedures

#### 2.4.1. Procedure 1

An aliquot of the sample containing between 25 and 250 ng of LFLX was diluted to 25-ml with 0.04 M acetate buffer solution (pH 4.0). The solution was first de-aerated by passage of a stream of nitrogen for 10 min. An accumulation potential of − 0.3 V was then applied to a fresh drop of mercury, while the solution was stirred at 2500 rpm throughout and accumulation time of 2 min. When the accumulation time was completed, the stirring was stopped and after a 15 s rest period, a differential pulse scan, with a scan rate of 5 mV $s^{-1}$ and a 50 mV pulse amplitude, was registered from − 1.00 to − 1.65 V.

A 0.04 M acetate buffer solution (pH 4.0), treated in the same way as the sample, was used as blank solution.

The calibration graph was constructed in the same way with LFLX solutions of known concentrations.

#### 2.4.2. Procedure 2

An aliquot of the sample containing between 200 and 1500 ng of LFLX was diluted to 25-ml with 0.04 M acetate buffer solution (pH 4.0). The solution was first de-aerated by passage of a stream of nitrogen for 10 min. An accumulation potential of − 0.3 V was then applied to a fresh drop of mercury, while the solution was stirred at 2500 rpm throughout and accumulation time of 30 s. The subsequent steps were as in Section 2.4.1.

## 3. Results and discussion

### 3.1. Cyclic voltammetry

Fig. 2 shows two sets of sequential cyclic voltammograms for 400 ng $ml^{-1}$ LFLX in 0.04 M acetate buffer solution (pH 4.0). When scanning the potential from − 1.00 to − 1.55 V (vs. Ag/AgCl) without any accumulation time a cathodic peak at about − 1.46 V was obtained because of reduction of dissolved LFLX, and no peak was observed on the anodic branch, indicating that the reduction of LFLX is irreversible. When accumulation at − 0.3 V was carried out a significantly large adsorptive stripping peak is observed as a result of the adsorption of the initial compound onto the mercury drop.

The spontaneous adsorption of LFLX can be used as an effective preconcentration step prior to the voltammetric quantitation of the drug. Fig. 3 shows voltammograms for 48 ng $ml^{-1}$ of LFLX in 0.04 M acetate buffer solution (pH 4.0) and supporting electrolyte only. Although quantitation at this level is not feasible without preconcentration, a well-defined peak was observed following preconcentration at − 0.3 V for 15 s.

### 3.2. pH dependence

The influence of pH on the LFLX reduction process was studied. The ip versus pH plot (Fig.

4) shows that the peak current is maximum in the pH interval 3.5–4.5. Different buffer solutions (acetate, monochloroacetate, phosphate and Britton–Robinson) were tested. Sodium acetate/acetic acid buffer solution (pH 4.0) was found to be the most successful. A 0.04 M concentration of the buffer was selected to obtain an adequate buffering capacity.

### 3.3. Effect of accumulation potential

The dependence of stripping peak current on the accumulation potential was evaluated over the range $-0.1$ to $-1.0$ V for 40 ng ml$^{-1}$ of LFLX

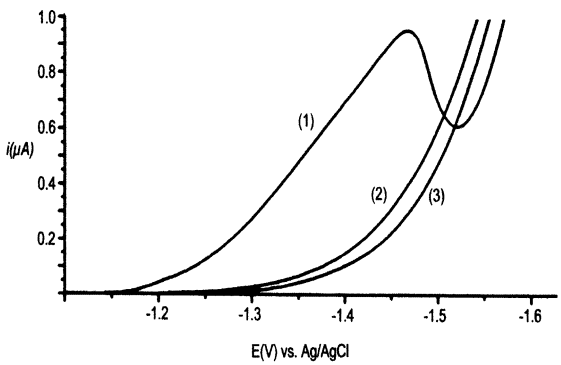


Fig. 3. Differential-pulse voltammograms of: (1) lomefloxacin with an accumulation time of 15 s; (2) lomefloxacin without accumulation time; (3) blank. [LFLX] = 48 ng ml$^{-1}$; pH 4.0 (acetate buffer); accumulation potential = $-0.3$ V.

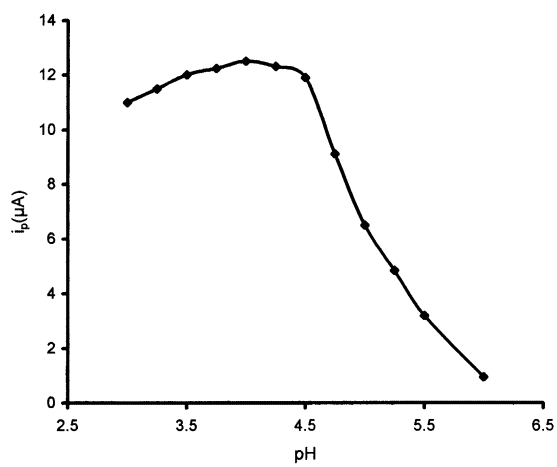


Fig. 4. Influence of pH on peak current ($i_p$).

Table 1
Analytical parameters

| | Calibration 1 | Calibration 2 |
|---|---|---|
| Intercept ($a$) (nA) | 23.37 | 84.32 |
| Slope ($b$) (nA ml ng$^{-1}$) | 84.76 | 11.93 |
| Correlation coefficient | 0.999 | 0.999 |
| Lack-of-fit test ($P$-value) | 0.23 | 0.22 |
| Linear dynamic range (ng ml$^{-1}$) | 1.0–10.0 | 8–60.0 |
| Linearity [1-RSD($b$)] (%) | 98.9 | 98.6 |
| Detection limit (ng ml$^{-1}$) | 0.3 | 2.3 |
| Quantification limit (ng ml$^{-1}$) | 1.0 | 7.6 |

at pH 4.0 for an accumulation period of 15 s. The results obtained shown that the Ip values are maxima for an accumulation potential of $-0.3$ V.

### 3.4. Effect of accumulation time

The dependence of stripping peak currents on accumulation time was studied at two concentration levels of LFLX: 40 and 6 ng ml$^{-1}$. Taking into account the results obtained, an accumulation time of 30 s and 2 min, corresponding to the maximum i.p. valued obtained at each concentration level studied, were chosen to evaluated the analytical parameters of the proposed method.

### 3.5. Instrumental parameters

Several instrumental parameters, such as drop size, stirring rate, scan rate and pulse amplitude, which directly affect to voltammetric response were optimised. The chosen working conditions were: a drop size of 3 (drop area ca. 0.52 mm$^2$), a stirring rate of 2500 rpm, a scan rate of 5 mV s$^{-1}$ and a pulse amplitude of 50 mV. The stripping currents were not modified when varying the rest period. The chosen value (15 s) is sufficient to allow the formation of a uniform concentration of the analyte in the mercury drop.

### 3.6. Analytical parameters

Two calibration graphs for the samples treated according to the procedures described above, were constructed. With 2 min accumulation time, the calibration graph is linear for the concentration range 1.0–10.0 ng $ml^{-1}$ of LFLX, and 30 s accumulation time the calibration graph is linear for the concentration range 8–60 ng $ml^{-1}$ of LFLX.

The lack-of-fit test [11] was used to check the linearity of the calibration graphs. Six replicates were used for each of five standards prepared to obtain the calibration graphs.

The IUPAC detection limits [12] found were 0.3 and 2.3 ng $ml^{-1}$ and the quantification limits [13] were 1.0 and 7.6 ng $ml^{-1}$.

The repeatability of the proposed method was determined. The precision was measured for a LFLX concentration of 5, 10 and 40 ng $ml^{-1}$ by performing ten independent determinations. The relative standard deviations (RSD) were 3.8, 1.1 and 0.8%, respectively.

The analytical parameters for two calibration graphs are summarised in Table 1.

Table 2
Results of recovery assays to check the accuracy of the proposed method for human urine samples

| Sample | Spiked[a] (μg $ml^{-1}$) | Found (μg $ml^{-1}$) | Recovery (%) |
|---|---|---|---|
| Human urine-1 | 25 | 24.58 | 98.3 |
| | 25 | 24.32 | 97.3 |
| | 25 | 25.55 | 102.2 |
| | 25 | 24.95 | 99.8 |
| | 50 | 50.14 | 100.3 |
| | 50 | 50.00 | 100.0 |
| | 50 | 48.75 | 97.5 |
| | 50 | 50.62 | 101.2 |
| | 75 | 76.17 | 101.6 |
| | 75 | 74.50 | 99.3 |
| | 75 | 76.25 | 101.7 |
| | 75 | 73.00 | 97.3 |
| Human urine-2 | 25 | 24.30 | 97.2 |
| | 25 | 25.15 | 100.6 |
| | 25 | 24.75 | 99.0 |
| | 25 | 24.63 | 98.5 |
| | 50 | 50.25 | 100.4 |
| | 50 | 48.75 | 97.5 |
| | 50 | 51.38 | 102.8 |
| | 50 | 50.62 | 101.2 |
| | 75 | 76.35 | 101.8 |
| | 75 | 75.75 | 101.0 |
| | 75 | 75.08 | 100.1 |
| | 75 | 74.40 | 99.2 |
| Human urine-3 | 25 | 25.59 | 102.4 |
| | 25 | 24.45 | 97.8 |
| | 25 | 24.90 | 99.6 |
| | 25 | 24.70 | 98.8 |
| | 50 | 49.81 | 99.6 |
| | 50 | 50.62 | 101.2 |
| | 50 | 51.00 | 102.0 |
| | 50 | 48.60 | 97.3 |
| | 75 | 72.90 | 97.3 |
| | 75 | 76.72 | 102.3 |
| | 75 | 75.50 | 100.7 |
| | 75 | 75.00 | 100.0 |

[a] Referred to original sample. Using the Student's $t$-test: $R = 99.7\%$; $s_R = 1.78$; $t(R) = 0.57$ ($P = 58\%$); critical value, 2.20 ($P = 5\%$) for human urine-1. $R = 99.9\%$; $s_R = 1.71$; $t(R) = 0.12$ ($P = 91\%$); critical value, 2.20 ($P = 5\%$) for human urine-2 and $R = 99.3\%$; $s_R = 1.87$; $t(R) = 0.17$ ($P = 87\%$); critical value, 2.20 ($P = 5\%$) for human urine-3.

### 3.7. Application and validation of the method

#### 3.7.1. Spiked samples

The proposed method was applied to the determination of LFLX in spiked human urine and serum samples using the standard addition method.

The human urine and serum samples were spiked at three different levels: 25, 50 and 75 μg $ml^{-1}$ for urine samples and 1, 3 and 5 μg $ml^{-1}$ for serum samples, respectively. The volume range of urine sample used was between 20 and 2 μl and the volume range of serum was between 100 and 30 μl.

Fig. 2 shows cyclic voltammograms of no-spiked serum (100 μl) with and without a 30 s accumulation time. From this figure, it is deduced that serum proteins do not competitively adsorb on the static mercury drop.

The validation of the proposed method for these samples was tested by using a recovery test (Student's $t$-test) [14,15]. Since the $P$-values calculated, 58% for urine-1, 91% for urine-2, 87% for urine-3, 25% for serum-1 and 41% for serum-2, are greater than 5%, so the null hypothesis appears to be valid, i.e. recovery is close to 100% (Tables 2 and 3).

Table 3
Results of recovery assays to check the accuracy of the proposed method for human serum samples

| Sample | Spiked[a] ($\mu g\ ml^{-1}$) | Found ($\mu g\ ml^{-1}$) | Recovery (%) |
|---|---|---|---|
| Human serum-1 | 1 | 0.98 | 98.0 |
| | 1 | 0.99 | 99.0 |
| | 1 | 1.00 | 100.0 |
| | 1 | 0.99 | 99.0 |
| | 3 | 2.83 | 100.3 |
| | 3 | 3.11 | 100.0 |
| | 3 | 3.01 | 97.5 |
| | 3 | 2.92 | 101.2 |
| | 5 | 4.94 | 98.8 |
| | 5 | 5.15 | 103.0 |
| | 5 | 4.89 | 97.8 |
| | 5 | 4.91 | 99.2 |
| Human serum-2 | 1 | 0.97 | 97.0 |
| | 1 | 1.02 | 102.0 |
| | 1 | 1.00 | 100.0 |
| | 1 | 1.02 | 102.0 |
| | 3 | 3.03 | 101.0 |
| | 3 | 3.08 | 102.7 |
| | 3 | 2.95 | 98.3 |
| | 3 | 2.87 | 95.7 |
| | 5 | 4.78 | 95.6 |
| | 5 | 4.91 | 98.2 |
| | 5 | 5.14 | 102.8 |
| | 5 | 4.82 | 96.4 |

[a] Referred to original sample. Using the Student's $t$-test: $R = 99.1\%$; $s_R = 2.50$; $t(R) = 1.22$ ($P = 25\%$); critical value, 2.20 ($P = 5\%$) for human serum-1. $R = 99.3\%$; $s_R = 2.77$; $t(R) = 0.87$ ($P = 41\%$); critical value, 2.20 ($P = 5\%$) for human serum-2.

### 3.7.2. Real samples

The proposed method was applied to determination of LFLX in human urine samples from healthy volunteers who received a single oral dose of 400 mg of lomefloxacin. The samples of individuals were collected for up to 24 h after administration of LFLX and the urinary volumes were recorded as well.

In this case, HPLC method proposed by Tan et al. [16,17] was used as a reference method.

The results obtained, summarised in Table 4, shown that both methods (AdSV and HPLC) yield values within the same range when tested using adequate statistical procedures [18].

## 4. Conclusions

A sensitive and practical differential-pulse adsorptive stripping voltammetric method for the determination of the antibacterian LFLX at ng $ml^{-1}$ level is presented. It was applied to human urine and serum samples with good recovery rates.

This is a rapid one step procedure which only requires a simple earlier sample treatment and to perform its voltammogram, so it is a inexpensive, simple and fast procedure which does not need an earlier separation of the analyte.

Although HPLC methods can be used to determine this drug in human urine and serum samples at $\mu g\ ml^{-1}$ level and usually, they can offer more accuracy than the proposed method, they are both more time consuming and expensive than the procedure here developed.

## Acknowledgements

This study was funded by the Spanish Intermin-

Table 4
Determination of lomefloxacin in human urine samples

| Sample | Lomefloxacin found[a] ($\mu g\ ml^{-1}$) | | $t$ | $P$(%)[b] |
|---|---|---|---|---|
| | Proposed method | HPLC method | | |
| Human urine-1 | $109 \pm 3$ | $112 \pm 2$ | 1.49 | 17.4 |
| Human urine-2 | $163 \pm 3$ | $161 \pm 3$ | 1.00 | 34.5 |
| Human urine-3 | $174 \pm 3$ | $176 \pm 2$ | 1.39 | 20.0 |

[a] Average value $\pm$ S.D. of five determinations.
[b] $P$ value of the two-sample comparison test. Urinary volume: 1.4 l for human urine-1; 1.6 l for human urine-2 and 1.3 l for human urine-3.

isterial Commission of Science and Technology (CAICYT) (Project No. PB96-1404). The authors are grateful to University of Zulia (Venezuela) for doctoral fellowships to L. Araujo and A. Prieto.

## References

[1] D. Dámaso, Antimicrobianos, Marketing Pharma S.A., Madrid, 1990, Chap. 24.
[2] T.L. Zhang, H.C. Zhao, L.P. Jin, Talanta 49 (1999) 77–82.
[3] G. Carlucci, A. Cilli, M. Liberato, P. Mazzeo, J. Pharm. Biomed. Anal. 11 (1993) 1105–1108.
[4] A.M. Shibl, A.F. Tawlik, S. El-Houfy, F.J. Al-Shammary, J. Clin. Pharm. Ther. 16 (1991) 353–359.
[5] C. Yin, Y. Wu, Yaowu Fenxi Zazhi 17 (1997) 371–373; Chem Abstr. 129 (1998) 140762.
[6] S.W. Sun, A.C. Wu, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 281–296.
[7] Y.M. Issa, F.M. Abdelgawad, M.A. Aboutable, H.M. Hussein, Anal. Lett. 30 (1997) 2071–2084.
[8] J.M. Pingarrón-Carrazón, P. Sánchez-Batanero, Química Electroanalítica, Fundamentos y Aplicación, Síntesis, Madrid, 1999, pp 292–296.
[9] Statgraphics, version 7.0 Plus, Manugistics Inc. and Statistical Graphics Corporation, USA, 1994.
[10] A.M. García-Campaña, L. Cuadros-Rodríguez, F. Alés-Barrero, M. Román-Ceba, J.L. Sierra-Fernández, TrAC 16 (1997) 381–385.
[11] Analytical Methods Committee, Analyst 119 (1994) 2363–2366.
[12] IUPAC, Nomenclature, Symbols, Units and Their Usage in Spectrochemical Analysis II, Spectrochim. Acta B 33B (1978) 242–245.
[13] Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry, Anal. Chem. 52 (1980) 2242–2249.
[14] K. Doerffel, Fresenius J. Anal. Chem. 348 (1994) 183–187.
[15] Analytical Methods Committee, Analyst 120 (1995) 29–34.
[16] L. Tan, D.K. Xu, Y. Diao, Y.S. Yuan, Yaoxue Xuebao, 28 (1993) 286–289; Chem. Abstr. 119 (1993) 40146j.
[17] F. Belal, A.A. Al-Majed, A.M. Al-Obaid, Talanta 50 (1999) 765–786.
[18] J.C. Miller, J.N. Miller, Statistics for Chemical Analysis, Addisson-Wesley Iberoamericana, Wilmington, 1993, pp. 40–63.