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Determination of Azithromycin by an Alternative FIA Amperometric Method for Different Pharmaceutical Applications

Valeria Pfaffen,[†] Sonia N. Faudone,[‡] Norma R. Sperandeo,[§] Silvia L. Cuffini,[‡,⊥] and Patricia I. Ortiz*[,†]

[†]INFIQC-CONICET, Departamento de Fisicoquímica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, X5000HUA Córdoba, Argentina.

[‡]Centro de Excelencia en Productos y Procesos de Córdoba CEPROCOR, Ministerio de Ciencia y Técnica de Córdoba, Álvarez de Arenales 230, X5004AAP Córdoba, Argentina.

[§]Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, X5000HUA Córdoba, Argentina

[⊥]Programa de Pós-Graduação em Farmácia, Universidade Federal de Santa Catarina, Florianópolis, UFSC, South Carolina, 88040-900 Florianópolis, Brazil

ABSTRACT: This work presents a fast and reliable electroanalytical procedure for the quantification of azithromycin (AZ) in different systems that couples flow injection analysis and amperometric detection (FIA-AM). The quantification was performed at a working potential of 1.20 V (vs Ag/AgCl/NaCl (3 M)) in 0.05 M phosphate buffer at a flow rate of 1.0 mL min^{-1}. Under these conditions a 1.00×10^{-6}–1.50×10^{-5} M linear range, $(5.8 \pm 0.4) \times 10^4$ C_{AZ} (μA M^{-1}) sensitivity and 4.44×10^{-7} M limit of detection are obtained. Also, this technique can be used without sample pretreatment and presents good reproducibility. The procedure was applied to the determination of the content of AZ in commercial tablets, to study the intrinsic dissolution rate of compacts of AZ dihydrate, and to determine the AZ release from a AZ hydrogel and carbomer. The method was statistically analyzed and proves to be reliable and robust. However, more remarkable is that FIA-AM technique presents better sensitivity and high sampling rate.

1. INTRODUCTION

Azithromycin (AZ) (9-deoxy-9a-aza-9a-methyl-9a-homoerythromycin A, Figure 1) is a member of the family of 15-membered macrolide antibiotics called azalides. It is used as an antibiotic in human medicine for the treatment or prophylaxis of a number of health problems such as pharyngitis, pneumonia, chronic bronchitis, bronchopneumonia, skin and soft tissue infections, and some sexually transmitted diseases.[1] AZ levels can be determined and studied through several methods[2−14] such as bioassay,[2] spectrophotometry,[3−5] spectrofluorometry,[6] high-performance liquid chromatography using amperometry,[7,8] fluorescence,[9,10] and UV[11] and mass spectrometry.[12−14] These methods can be applied to the detection of AZ in various samples, but they are generally time-consuming and/or complicated. AZ can also be assayed and studied by using electrochemical methods. The oxidative behavior of AZ at different electrodes such as glassy carbon electrode, handmade carbon paste electrode, and gold electrode was used for the determination of AZ.[15−17] A polymeric membrane electrode has been developed for the determination of AZ with the limit of detection (LOD) 1.54 μg mL^{-1}.[18]

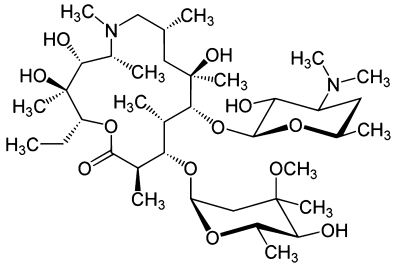

Figure 1. Chemical structure of azithromycin.

To the best of our knowledge, there are only three reported FIA methods for the determination of AZ in pharmaceutical formulations.[4,19,20] Song et al.[19] have reported a flow-injection chemiluminescence method for the determination of AZ in pharmaceutical preparations, human urine, and serum. However, despite its high sampling rate, excellent analytical sensitivity, and relative low cost, this method presents drawbacks, such as the complexity of the chemiluminescence mechanism of the system utilized, the critical detection of the chemiluminescence signal within a short and strictly defined period of time, and the efficiency of the proposed reaction only at pH 12.4. A flow-injection procedure for the spectrophotometric determination of AZ in pharmaceutical formulations has also been proposed[4] on the basis of the reaction of AZ with tetrachloro-*p*-benzoquinone (*p*-chloranil) accelerated by hydrogen peroxide and conducted in a methanol medium, producing a purple-red compound (λ_{max} = 540 nm). Although the method is relatively simple, the procedure is performed in methanol and cannot be used to analyze samples other than pharmaceutical formulations. Finally, an FIA amperometric method for the determination of AZ in pharmaceutical formulations (AZ

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dihydrate) has also been proposed[20] that presents good analytical sensitivity; however, the main disadvantage is a low sampling rate due to a cleaning cycle that is included.

In this work a sensitive, simple, and reproducible procedure for the quantification of AZ in different samples that is based on a flow injection system with amperometric detection (FI-AM) is described. The analytical parameters were determined. This procedure was applied to the determination of the AZ content of commercial tablets, to the evaluation of the intrinsic dissolution rate of AZ dihydrate compacts, and to study the AZ release from AZ hydrogels and carbomer as examples of its application as a fast method. This procedure is intended to be an alternative, simple, and fast tool for AZ quantification in different systems. Therefore, the discussion is centered on the analytical procedure and the advantages of using the proposed approach in such systems.

2. EXPERIMENTAL SECTION

2.1. Apparatus. The flow injection analysis system has been previously described.[21] It consists of a peristaltic pump (Gilson Miniplus 3), adjusted to 1.0 mL min^{-1} flow rate, and a Rheodyne type 7125 injection valve with a 20 μL sample injection volume. Quantifications were performed in an electrochemical flow cell with a stainless steel auxiliary electrode block, a 51 μM TG-2 M Teflon cell gasket (MF-1046 Bioanalytical Systems), and an LC-4C amperometric detector (BAS). The working potential was applied to the same glassy carbon disk electrode vs a Ag/AgCl/NaCl 3 M reference electrode (RE-4 BAS), allowing the current to decay to its stationary value after each measurement.

2.2. Solutions and Reagents. Stock solutions (2×10^{-2} M) were prepared in 0.05 M phosphate buffer of pH 7.0 by dissolving accurately weighed amounts of AZ dihydrate (Parafarm, Argentine) in purified water. Aqueous standard solutions were prepared by diluting the stock solution with phosphate buffer. Water was purified in a Milli-Q system (Millipore, U.S.A.). All other reagents were of analytical grade. The 0.05 M phosphate buffer solutions (Barker) were prepared by weighing appropriate amounts of the compound in pure water every day.

2.3. Studied Systems. In order to test the proposed method, three different systems were analyzed: (1) the determination of the content of AZ of a commercial pharmaceutical formulation, (2) the evaluation of the intrinsic dissolution rate of compacts of AZ dihydrate, and (3) the study of the kinetic of liberation of AZ from an AZ hydrogel and carbomer, designated as C_{934}-AZ_{40}.

In the first case, three tablets of a commercial formulation labeled as containing 500 mg of AZ [corresponding to 524.10 mg AZ dihydrate (Doyle, 57164, Raffo Laboratory, Argentina)] were weighed and crushed to a fine powder in an agate mortar. Accurately weighed amounts of this powder were dissolved in 0.05 M phosphate buffer, pH 6.8, giving a final concentration of 1×10^{-4} M AZ. This solution was further diluted to fit the linear concentration range. Each determination was carried out six times, and the amount of AZ was determined by the simple calibration method. No additional sample pretreatment was required. In the second system, the disk intrinsic dissolution rate (DIDR) of AZ dihydrate was determined by the rotating disk method using a USP rotating disk apparatus (die cavity diameter/area: 0.8 cm/0.5 cm^2).[22] The DIDR (J) is defined as the dissolution rate of pure substances under the condition of a constant surface area, temperature, stirring rate, and pH[23] and is calculated by the following:

$$J = V\frac{dc}{dt}\frac{1}{A}$$

where J is the DIDR, V is the volume of the dissolution medium, c is the concentration, A is the area of the drug disk, and t is the time. For the disk preparation, 250 mg of AZ dihydrate was compressed in the rotating disk die at 200 kg force for 1 min, using a hydraulic press (SHIMADZU, SSP-10A). DIDR studies were conducted on a Hanson SR6 dissolution tester (Hanson Research, CA, U.S.A.), using 500 mL of 0.05 M deaereated phosphate buffer, pH 6.8, at 37 ± 0.5 °C and a 50 rpm rotation speed. For all experiments, aliquots (3 mL) were withdrawn with replacement at appropriate time intervals (5 min). Two independent measurements were performed.

Finally, the third system analyzed was evaluation of AZ release from aqueous dispersions. Experiments were performed in a modified Franz diffusion assembly at 37 ± 1 °C. Semipermeable acetate cellulose membrane (Sigma 12000) was placed between the donor and receptor compartments. Twenty milliliters of hydrogel was placed in the upper compartment while the receptor compartment was filled with 300 mL of phosphate buffer, pH 6.8, and stirred at 260 rpm with a Teflon-coated magnetic stirring bar. At selected time intervals aliquots (3 mL) were withdrawn and replaced by the same volume of fresh and prewarmed receptor medium. The assays were done in triplicate. Hydrogel preparation was described elsewhere,[24,25] but briefly AZ was used to neutralize 40% of the carboxylic groups of a 0.25% aqueous dispersion of carbomer and AZ (C_{934}-AZ_{40}).

3. RESULTS AND DISCUSSION

3.1. Method Optimization and Operational Parameters. Cyclic voltammograms of standard AZ solutions at different pH values were already obtained[20] indicating that the compound is electroactive. However in that proposed method a surface regeneration cycle was proposed, that made the whole procedure rather slow and troublesome. In order to obtain a faster method an alternative was analyzed. Figure 2 shows the hydrodynamic voltammogram for 1.0×10^{-5} M AZ in the 0.80−1.30 V potential range. In this case a current plateau is never reached. For that reason, a working potential of 1.20 V was selected, as at this potential value the highest current is obtained, and no oxygen evolution is observed.

Figure 3 shows the current dependence on the injection frequency. Data were empirically obtained from an independent series of 15 successive injections of 1.0×10^{-5} M AZ, in the 30−240 injections per hour range (at a 1.00 mL min^{-1} flow rate). As can be observed, no significant variations with the injection frequency is obtained; hence, a frequency of 120 injections h^{-1} was selected, as a good baseline is observed and allows an important number of determinations.

The current dependence on the flow rate is shown in Figure 4 for 1.00×10^{-5} M AZ solution in the 0.25−1.50 mL min^{-1} range. As it can be observed for flow rates lower than 0.75 mL min^{-1} or higher than 1.11 mL min^{-1}, there is a smooth current increase, with a minimum and constant value at ∼1.00 mL min^{-1}, and so this is the chosen value as there is a good and stable current signal.

The electrode response stability was analyzed by consecutive injections of 1.00×10^{-5} M AZ solutions during 1110 s (not

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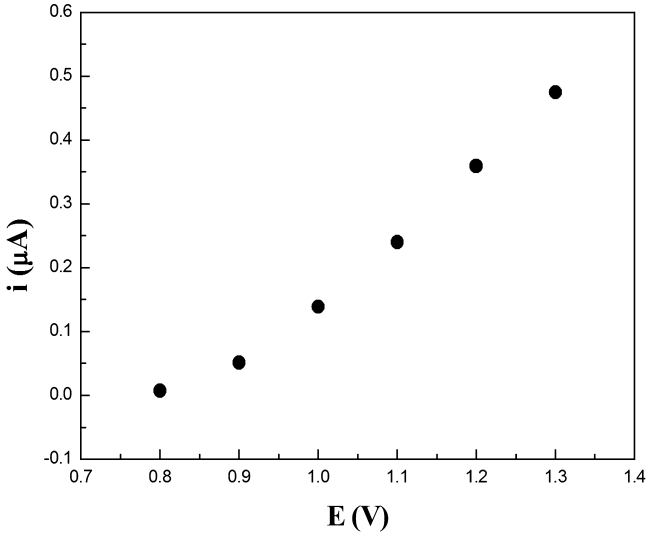

Figure 2. Hydrodynamic voltammogram for azithromycin 1.00×10^{-5} M in phosphate buffer 0.05 M (pH 7.00) at 1.00 mL min^{-1} flow rate and with a 20 μL injection volume.

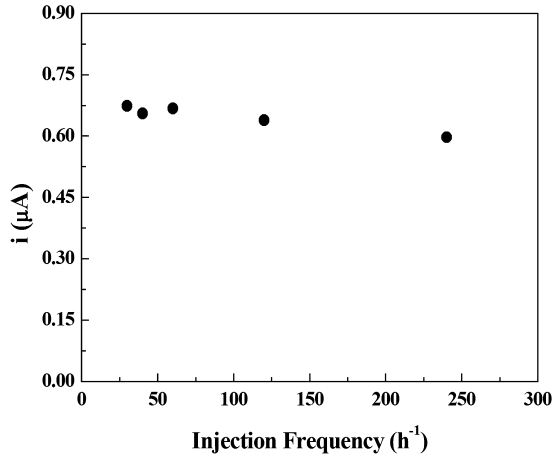

Figure 3. Effect of injection frequency in response to a solution of azithromycin 1×10^{-5} M, 1.200 V, 1.00 mL min^{-1}.

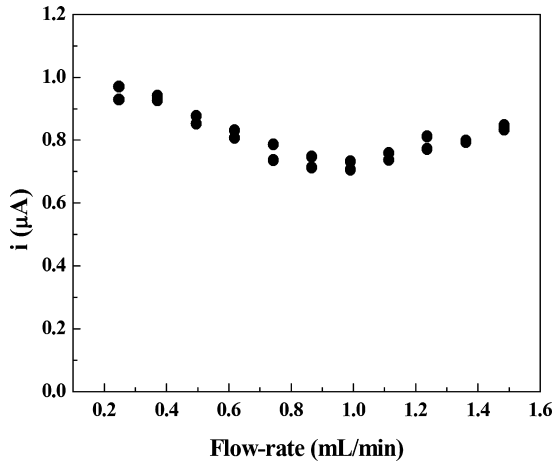

Figure 4. Flow-rate dependence on the flow injection response. Azithromycin concentration, 1.00×10^{-5} M in phosphate buffer 0.05 M (pH 7.00) at 1.200 V and with a 20 μL injection volume.

shown). There is a slight current decrease in the first 200 s (~15%), and then the current signal remains practically constant, indicating that the electrode can be used in an FIA-AM system.

After the FIA-AM optimization, the analytical characteristics such as linearity range, detection limit, precision, accuracy, and matrix effect were analyzed, prior to its use in different real systems.

The analytical method linearity and reproducibility were obtained by performing 10 AZ calibration curves on different days and with different solutions from which the corresponding regression curves were obtained. Calibration curves were performed in the 1.00×10^{-6}–7.00×10^{-5} M concentration range; however, for concentration values higher than 1.50×10^{-5} M, deviations from linearity are observed. For that reason the concentration range was limited to 1.00×10^{-6}–1.50×10^{-5} M where lineal dependences are obtained. The average and standard deviation for the slope and the ordinate of all the calibration curves were calculated, and the obtained equation is

$$i_p\,(\mu A) = (5.8 \pm 0.4) \times 10^4\, C_{AZ}\,(\mu A\, M^{-1}) + (0.014 \pm 0.009)\,(\mu A)$$

The limit of detection (LOD) was calculated on the basis of the standard deviation of the response (δ) and the slope (S) of the calibration curves at the levels approaching the limits according to the equation LOD = $3.3(\delta/S)$, being 4.44×10^{-7} M.

The precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was determined by injecting standard solution at three levels of concentration, 5.00×10^{-6}, 1.00×10^{-5}, and 1.50×10^{-5} M (six replicates each). In Table 1 the obtained results, expressed in terms of the coefficient of variation (CV %), for five successive AZ records are shown. Interday precision was assessed by injecting the same three concentrations over three consecutive days. As can be observed, CV values of less than 6% were obtained, indicating that the repeatability of the proposed method is acceptable.

Table 1. Analysis of Known Concentration Samples

theor. conc (M)	exp'l conc (M)	CV[a] (%)	p value
5.00×10^{-6}	5.10×10^{-6}	6	0.5198
1.00×10^{-5}	1.04×10^{-5}	3	0.1472
1.50×10^{-5}	1.51×10^{-5}	1	0.3836

[a]CV = coefficient of variation.

Accuracy was determined by analyzing a known sample, comparing the measured value with the true value, and determining the percentages of recovery at three levels of concentration in two different situations. Recovery data obtained were within the 98–104% range (Table 2). Applying the t test, the experimental mean was not significantly different from the true value with 95% confidence ($p > 0.05$) (Table 1).

Matrix interference can introduce systematic errors on the analytical determination. The relative systematic errors can be detected by applying the standard addition calibration method to different real samples. Thus, a comparison between the slopes of the standard addition calibration lines and a standard calibration line was carried out. If the matrix does not interfere, both lines must have the same slope. Figure 5 shows the calibration slope lines obtained with the proposed method and with the standard addition method applied to a Doyle AZ

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Table 2. Recovery Values Obtained from Standard Additions of the Analyte to a Doyle AZ Sample

sample	added amount (g/tablet)	recovery ± SD[a] (%)
AZ Doyle	0.2103	98 ± 5
	0.4392	104 ± 3
	0.5906	102 ± 4

[a]Mean values and standard deviation of three determinations.

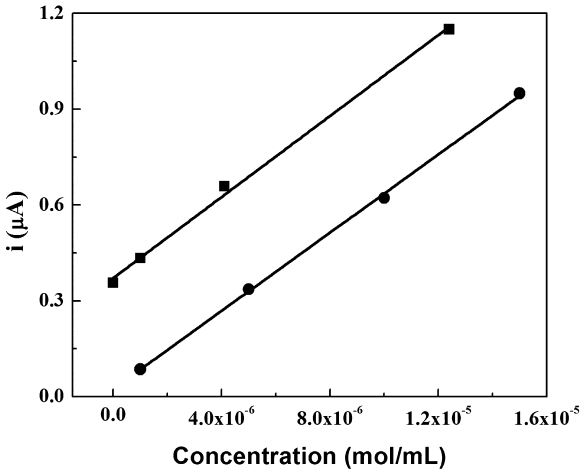

Figure 5. Calibration curves of proposed method (•) and standard addition method applied to a Doyle AZ sample (■), 1.200 V, 1.00 mL min^{-1}.

sample. Slope comparison was performed by applying the *t* test, slopes were not significantly different from the true value with 95% confidence ($p > 0.05$) (not shown).

3.2. Analytical Application to Real Systems. *3.2.1. Azithromycin Determination in Commercial Tablets.* The proposed amperometric procedure was successfully applied for the determination of AZ in a commercial pharmaceutical formulation without interference from its excipients on the basis of the average of six replicates. The average AZ content is 0.51 ± 0.03 g/tablet, that when statistically compared with *t* test with the nominal value (0.5241 g/tablet) no significant differences were observed with 95% confidence regarding accuracy and precision as revealed by the $p = 0.3711$ value ($p > 0.05$).

3.2.2. Azithromycin Intrinsic Dissolution Rate Determination. The DIDR of AZ dihydrate was determined at 37 °C in buffer phosphate pH 6.8 at 50 rpm. Figure 6 shows the mean dissolution profile for this sample expressed as mg mL^{-1} vs time (min). As it can be observed, there is a lineal dependence between the AZ dihydrate dissolved amount expressed as mg mL^{-1} and time, in good agreement for a dissolution process where the humid drug area remains constant, indicating that the developed FIA-AM method can be used to evaluate the intrinsic dissolution behavior of this solid-state form (AZ dihydrate). The slope of the dissolution profile was used to derive the *J* of AZ dihydrate, which was found to be 0.169 ± 0.004 mg cm^{-2} min^{-1}.

3.2.3. Azithromycin Release Kinetics from Hydrogels of Carbomer. The proposed method was also used to evaluate the release kinetics of AZ from hydrogels of carbomer and AZ. The samples were provided and prepared by S. Esteban, from Departamento de Farmacia, Facultad de Ciencias Químicas-UNC. In Figure 7 the AZ release profile from the hydrogel of

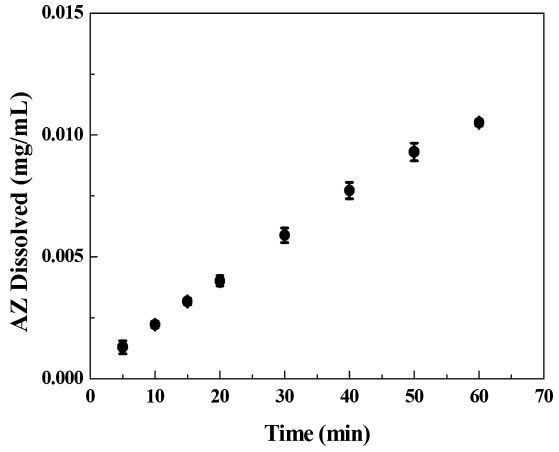

Figure 6. Mean intrinsic dissolution profile ($n = 2$) of azithromycin dihydrate (250 mg) in phosphate buffer pH 6.8 at 37 °C and 50 rpm.

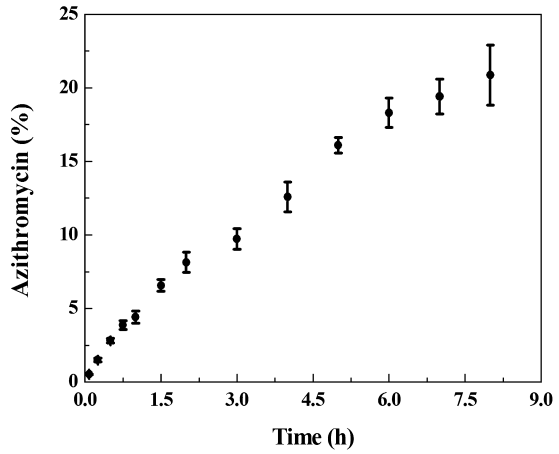

Figure 7. Azithromycin release profile from (C_{934}-AZ_{40}).

carbomer and AZ (C_{934}-AZ_{40}) is presented. The amount in percentage of AZ released was 20.8%, being the obtained amount in good agreement with the value obtained by HPLC with UV detection (21.79%),[25] indicating that the proposed method can also be used for this type of determination.

4. CONCLUSIONS

A FIA-AM system for AZ determination has been optimized. The procedure provides a simple and fast analysis and presents wide measurement range and high sensibility. FIA-AM shows equivalent analytical parameters in comparison with other common techniques (UV−vis) for AZ quantification. However, FIA-AM shows three major advantages: absence of the sample pretreatment step, increased rate of analysis, and lower limit of detection.

This method has been successfully employed for the quantification of AZ in three different systems: commercial tablets labeled as containing 500 mg of AZ, compacts of AZ dihydrate for DIDR studies, and AZ release from a hydrogel of carbomer and AZ. In the first case, the commercial tablets can be analyzed without any further pretreatment, while in the other two cases the good precision and appropriate low limit of detection allowed the quantitative, easy, fast, and reliable measure of dissolved and released amounts of AZ from compacts of pure AZ dihydrate and hydrogels of AZ and carbomer, respectively.

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AUTHOR INFORMATION

Corresponding Author

*Tel.: +54-351-4334169. Fax: +54-351-4334188. E- mail: portiz@fcq.unc.edu.ar.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Kremer, C. J. Azithromycin: A new macrolide. *Primary Care Update OB/GYNS* **2002**, *9*, 174−175.

(2) Turcinov, T.; Pepeljnjak, S. Azithromycin potency determination: Optimal conditions for microbiological diffusion method assay. *J. Pharm. Biomed. Anal.* **1998**, *17*, 903−910.

(3) De Paula, C. E. R.; Almeida, V. G. K.; Cassella, R. J. Novel spectrophotometric method for the determination of azithromycin in pharmaceutical formulations based on its charge transfer reaction with quinalizarin. *J. Braz. Chem. Soc.* **2010**, *21*, 1664−1671.

(4) Rufino, J. L.; Pezza, H. R.; Pezza, L. Flow-injection spectrophotometric determination of azithromycin in pharmaceutical formulations using *p*-chloranil in the presence of hydrogen peroxide. *Anal. Sci.* **2008**, *24*, 871−876.

(5) Rachidi, M.; Elharti, J.; Digua, K.; Cherrah, Y.; Bouklouze, A. New spectrophotometric method for azithromycin determination. *Anal. Lett.* **2006**, *39*, 1917−1926.

(6) Khashaba, P. Y. Spectrofluorimetric analysis of certain macrolide antibiotics in bulk and pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **2002**, *27*, 923−932.

(7) Gandhi, R.; Kaul, C. L.; Panchagnula, R. Validated LC method for in vitro analysis of azithromycin using electrochemical detection. *J. Pharm. Biomed. Anal.* **2000**, *23*, 1073−1078.

(8) Taninaka, C.; Ohtani, H.; Hanada, E.; Kotaki, H.; Sato, H.; Iga, T. Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. *J. Chromatogr. B* **2000**, *738*, 405−411.

(9) Bahrami, G.; Mirzaeei, S.; Kiani, A. High performance liquid chromatographic determination of azithromycin in serum using fluorescence detection and its application in human pharmacokinetic studies. *J. Chromatogr., B* **2005**, *820*, 277−281.

(10) Wilms, E.; Trumpie, H.; Veenendaal, W.; Touw, D. Quantitative determination of azithromycin in plasma, blood and isolated neutrophils by liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl-chloride and fluorescence detection. *J. Chromatogr., B* **2005**, *814*, 37−42.

(11) Yang, Z. Y.; Wang, L.; Tang, X. Determination of azithromycin by ion-pair HPLC with UV detection. *J. Pharm. Biomed. Anal.* **2009**, *49*, 811−815.

(12) Choemunng, A.; Na-Bangchang, K. An alternative liquid chromatography-mass spectrometric method for the determination of azithromycin in human plasma and its application to pharmacokinetic study. *J. Liq. Chromatogr. Relat. Technol.* **2010**, *33*, 1516−1528.

(13) Liu, F.; Xu, Y.; Huang, J.; Gao, S.; Guo, Q. Sensitive liquid chromatography/mass spectrometry assay for the quantification of azithromycin in human plasma. *Biomed. Chromatogr.* **2007**, *21*, 1272−1278.

(14) Chen, B.; Liang, Y.; Chen, X.; Liu, S.; Deng, F.; Zhou, P. Quantitative determination of azithromycin in human plasma by liquid chromatography-mass spectrometry and its application in a bioequivalence study. *J. Pharm. Biomed. Anal.* **2006**, *42*, 480−487.

(15) Nigovic, B.; Simunic, B. Voltammetric assay of azithromycin in pharmaceutical dosage forms. *J. Pharm. Biomed. Anal.* **2003**, *32*, 197−202.

(16) Farghaly, O. A. E.; Mohamed, N. A. L. Voltammetric determination of azithromycin at the carbon paste electrode. *Talanta* **2004**, *62*, 531−538.

(17) Avramov Ivic, M. L.; Petrovic, S. D.; Mijin, D. Z.; Zivkovic, P. M.; Kosovic, I. M.; Drljevic, K. M.; Jovanovic, M. B. Studies on electrochemical oxidation of azithromycin and Hemomycin® at gold electrode in neutral electrolyte. *Electrochim. Acta* **2006**, *51*, 2407−2416.

(18) Rachidi, M.; Elharti, J.; Digua, K.; Cherrah, Y.; Bouklouze, A. New polymeric membrane electrode for azithromycin determination. *Anal. Lett.* **2007**, *40*, 53−66.

(19) Song, Z.; Wang, C. Ultrasensitive assay of azithromycin in medicine and bio-fluids based on its enhanced luminol-H_2O_2 chemiluminescence reaction using flow injection technique. *Bioorg. Med. Chem.* **2003**, *11*, 5375−5380.

(20) Palomeque, M. E.; Ortiz, P. I. New automatized method with amperometric detection for the determination of azithromycin. *Talanta* **2007**, *72*, 101−105.

(21) Pfaffen, V.; Ortiz, P. I. Alternative method with amperometric detection for ranitidine determination. *Ind. Eng. Chem. Res.* **2010**, *49*, 4026−4030.

(22) The United States Pharmacopeia and National Formulary USP 32-NF 27. The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2009.

(23) Yu, L. X.; Carlin, A. S.; Amidon, G. L.; Hussain, A. S. Feasibility studies of utilizing disk intrinsic dissolution rate to classify drugs. *Int. J. Pharm.* **2004**, *270*, 221−227.

(24) Esteban, S. L.; Manzo, R. H.; Alovero, F. L. Azithromycin loaded on hydrogels of carbomer: Chemical stability and delivery properties. *Int. J. Pharm.* **2009**, *366*, 53−57.

(25) Esteban, S. L. Sistemas poliméricos portadores de macrólidos: diseño y evaluación. (Engl. transl. Polymeric systems as macrolide carriers: design and evaluation.) Master's Thesis, Universidad Nacional de Córdoba: Córdoba, Argentina, 2007.