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Journal of Chromatographic Science, Vol. 48, November/December 2010

Degradation Kinetics of Telithromycin Determined by HPLC Method

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Abstract

The degradation kinetics of the antibiotic telithromycin using a stability-indicating high-performance liquid chromatography (HPLC) method is demonstrated. The photodegradation is performed by UVC lamp-254 nm (15W), installed in a chamber internally coated with mirrors, where telithromycin solutions prepared from coated tablets are placed in quartz cells. To promote oxidation, the reaction between the telithromycin solution and 3% hydrogen peroxide solution is carried out. The kinetics parameters of order of reaction and the rate constants of the degradation are determined for both conditions. The degradation process of telithromycin can be described by first-order kinetics under both experimental conditions used in this study. The results reveal the photo and oxidation lability of the drug and confirm the reliability of HPLC method for telithromycin in the presence of its degradation products.

Introduction

Telithromycin (Figure 1) 3-De[(2,6-dideoxy-3-*C*-methyl-α-Lribohexoppyranosyl)oxy]-11,12-dideoxy-6-*O*-methyl-3-oxo-12,11-[oxycarbonyl[[4-[4-(3-pyridinil)-1*H*-imidazol-1-1-yl] butyl]imino]] erythromycin; [191114-48-4]; $C_{43}H_{65}N_5O_{10}$; molecular weight 812.00; is the first antibiotic belonging to a new class of 14-membered ring macrolides, named ketolides, to achieve clinical use. This new addition to the macrolide-lincosamide-streptogramin B (MLS_B) group was developed specifically for the treatment of community-acquired respiratory tract infections (1,2).

The discovery of ketolides, derived from erythromycin incorporating a C-3 ketone modification, revealed a class of compounds with excellent activity against some macrolide-resistant bacteria, especially clinically important respiratory tract pathogens, such as *Streptococcus pneumoniae.* The positive results showed by the use of ketolides could be responsible for the increase of macrolide antibiotics research in the pharmaceutical industry (3).

All macrolide structures and their ketolide-derivatives are based on a macrolactone ring, being the most therapeutically relevant macrolides comprising a 14-, 15- or 16-membered ring. Erythromycin A, a natural antibiotic isolated from *Streptomyces erythreus* consists of a 14-membered lactone ring with two attached sugar groups: L-cladinose at C3 and desosamine at C5.

Telithromycin is structurally differentiated from the macrolides in three ways, which are associated with specific improvement in antimicrobial properties (4,5).

Assays reported in the literature for the determination of telithromycin in biological fluids include high-performance liquid chromatography-mass spectrometry (6), liquid chromatography (LC) with fluorescence detection (7), and microbiological assays (8). Based on results, the authors have made a critical analysis of in vitro and in vivo activities, pharmacokinetic, and pharmacodynamic considerations concerning the use of this new agent for the treatment of respiratory infections. Other researchers have described a sensitive method for the examination of 20 macrolides and ketolides in bulk, using an LC method with a volatile mobile phase, allowing the recovery and subsequent analysis of these antibiotics (8). The quantitation of telithromiycin in the presence of its degradation products has been studied. Recently, a microbiological assay and LC method were performed for quantitative determination of the antibiotic in the pharmaceutical dosage form and in a sample submitted to degradation conditions. A microbiological assay applying cylinder-plate method was performed

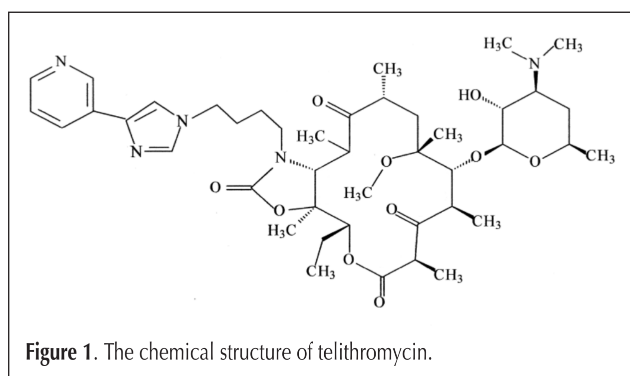

Figure 1. The chemical structure of telithromycin.

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and described in a previous study (9). The microbiological assay reveals subtle changes not demonstrable by chemical methods. This assay makes the evaluation of the potency possible, which is very important in the analysis of antibiotics (10).

A selective and reliable high-performance liquid chromatographic (HPLC) method was developed and validated according to International Conference on Harmonization (ICH) Q2 (R1) requirements (11), for the quantitative evaluation of the drug and in the presence of its degradation products (12).

Preliminary stability investigations carried out by our research group revealed that telithromycin undergoes degradation upon exposure to light and oxidizing agent. Its light and oxidation lability were established by forced degradation testing (stress testing). ICH guideline presents the standard conditions for stability testing and requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance in a pharmaceutical preparation. Following the recommendations of this guide, the light testing should be an integral part of stress testing. Besides, a stability-indicating method is necessary to quantify the drug in the presence of its degradation products. The method should be capable of resolving and detecting photolytic degradants that appear during the study (13). The purpose of this work was to establish the effect of oxidation and light on the decomposition of this antibiotic; to determine the kinetics of degradation describing the concentration changes as a function of time; and to estimate kinetics parameters such as apparent order degradation rate constant (k) and $t_{90\%}$ (time where 90% of original concentration of the drug is left unchanged), using a stability-indicating HPLC method.

Experimental

Chemicals

HPLC-grade methanol, sodium hydroxide, hydrochloric acid, hydrogen peroxide, potassium dihydrogen phosphate and orthophosphoric acid (reagent grade) were purchased from Merck (Darmstadt, Germany). Distilled water purified by a Millipore Milli-Q UF-Plus apparatus (Millipore, Bellerica, MA) was used to prepare the mobile phase.

Telithromycin film-coated tablets were claimed to contain 400 mg (as the anhydrous base) of the drug and the following inactive ingredients: corn starch, croscarmellose sodium, hypromellose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylene glycol, povidone, red ferric oxide, talc, titanium dioxide, and yellow ferric oxide.

The telithromycin reference standard (99.3%), the pharmaceutical dosage form and the excipients were supplied by Aventis Pharma (São Paulo, Brazil).

Chromatography

The stability-indicating HPLC method was developed and validated using a Shimadzu LC equipped with a model LC-10 ADvp binary pump, SIL-10 ADvp autosampler and model SPD-M10 Avp UV detector (Kyoto, Japan).

Detection was made at 265 nm. An SCL-10 Avp system controller and CLASS-VP chromatography software were used. A CTO-10 Acvp oven was used to keep the temperature at 50°C. The stationary phase was an Ace RP-18 octadecyl silane column (250 mm × 4.6 mm, 5 µm) and it was operated at 40°C. The mobile phase was composed of methanol and M 0.067 potassium monobasic phosphate buffer adjusted to pH 4.0 with orthophosphoric acid (55:45, v/v). It was prepared daily, filtered through a 0.45-µm membrane filter, and degassed using the degasser of the chromatographic system prior to use. The flow rate of the mobile phase was 1 mL/min and the injection volume was 20 µL.

Decomposition studies

Photodegradation study

The light source used was a UV fluorescent lamp model Ecolume, 30W, emitting radiation at 254 nm, fixed to a chamber in a horizontal position. The chamber was internally coated with mirrors in order to distribute the light uniformly. The effect of light was studied exposing the sample solutions in 1-cm quartz cells. The temperature was controlled in the chamber. A stock solution (1 mg/mL) in methanol was prepared from the coated tablets. The stress degradation study was performed exposing the solutions contained in the quartz cells in the chamber. The samples were positioned horizontally to provide maximum area of exposure to the light source. Considering the UV absorption of telithromycin, the irradiation was carried out at 254 nm at different time intervals (0, 1, 2, 3, 4, 5, 6, 7, and

Table I. Results of the Residual Concentration of Telithromycin Solutions after Photodegradation and Oxidative Degradation, Using the HPLC Method

	Time (h)	Sample concentration (µg/mL)	Measured concentration* (µg/mL)	Relative Standard Deviation
Photodegradation	0	40.00	40.00 (100%)	1.88
	1		32.86 (82.16%)	2.44
	2		21.08 (52.71%)	1.75
	3		16.13 (40.34%)	2.22
	4		14.77 (36.93%)	2.65
	5		10.44 (26.10%)	1.50
	6		9.52 (23.78%)	2.75
	7		6.78 (16.96%)	1.98
	8		5.73 (14.31%)	2.32
Oxidative degradation	0	40.00	40.00 (100%)	0.76
	1		21.53 (53.83%)	1.64
	2		18.10 (45.26%)	0.95
	3		10.61 (26.53%)	0.67
	4		11.11 (27.78%)	1.93
	5		5.32 (13.31%)	1.50
	6		5.22 (13.05%)	1.75
	7		3.79 (9.47%)	2.23
	8		3.55 (8.88%)	2.05

* Each value is the mean of three analysis.

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8 h). In order to evaluate the contribution of thermally induced change to the total change, protected samples, wrapped in aluminium foil, were used as dark controls. Three samples were analyzed for each time interval. After each time point, the samples were diluted with the mobile phase to give final concentration of 40 µg/mL). Standard solutions prepared in methanol were diluted with the mobile phase in the concentration of 40 µg/mL to the quantitation of the drug then assayed by HPLC. All solutions were injected in triplicate. Placebo solutions were prepared the same way in order to verify the influence of the excipients in the degradation process.

Oxidative degradation study

To promote oxidation, preliminary trials were done using the reaction between the telithromycin solutions and 1% and 3% hydrogen peroxide solution.

A stock solution (1 mg/mL) was prepared when the equivalent of 50 mg of telithromycin was transferred to a volumetric flask of 50 mL, solubilized with 5 mL of methanol and the volume was completed with hydrogen peroxide 1% and 3%, respectively. The samples were wrapped in aluminium foil and left at room temperature.

The samples were analyzed at different time intervals 0, 1, 2, 3, 4, 5, 6, 7, and 8 h of exposure. Three samples were analyzed for each time interval. After each time, the samples were diluted with the mobile phase to give final concentration of 40 µg/mL. Standard solutions, prepared in methanol, were diluted with the mobile phase in the concentration of 40 µg/mL to the quantitation of the drug, then were assayed by HPLC. All solutions were injected in triplicate. Placebo solutions were prepared similarly in order to verify the influence of the excipients in the degradation process.

Kinetics calculations

The degradation rate kinetics of telithromycin were determined by plotting the concentration of the drug remaining versus time (zero-order process), log of the concentration of the drug versus time (first-order process), and reciprocal of the concentration of the drug versus time (second-order process). Regression coefficients (*r*) were obtained, and the best fit observed indicates the reaction order. The kinetics parameters such as apparent order degradation rate constant (k), and t_{90} (time where 90% of original concentration of the drug is left) were obtained. The kinetics model can be represented as:

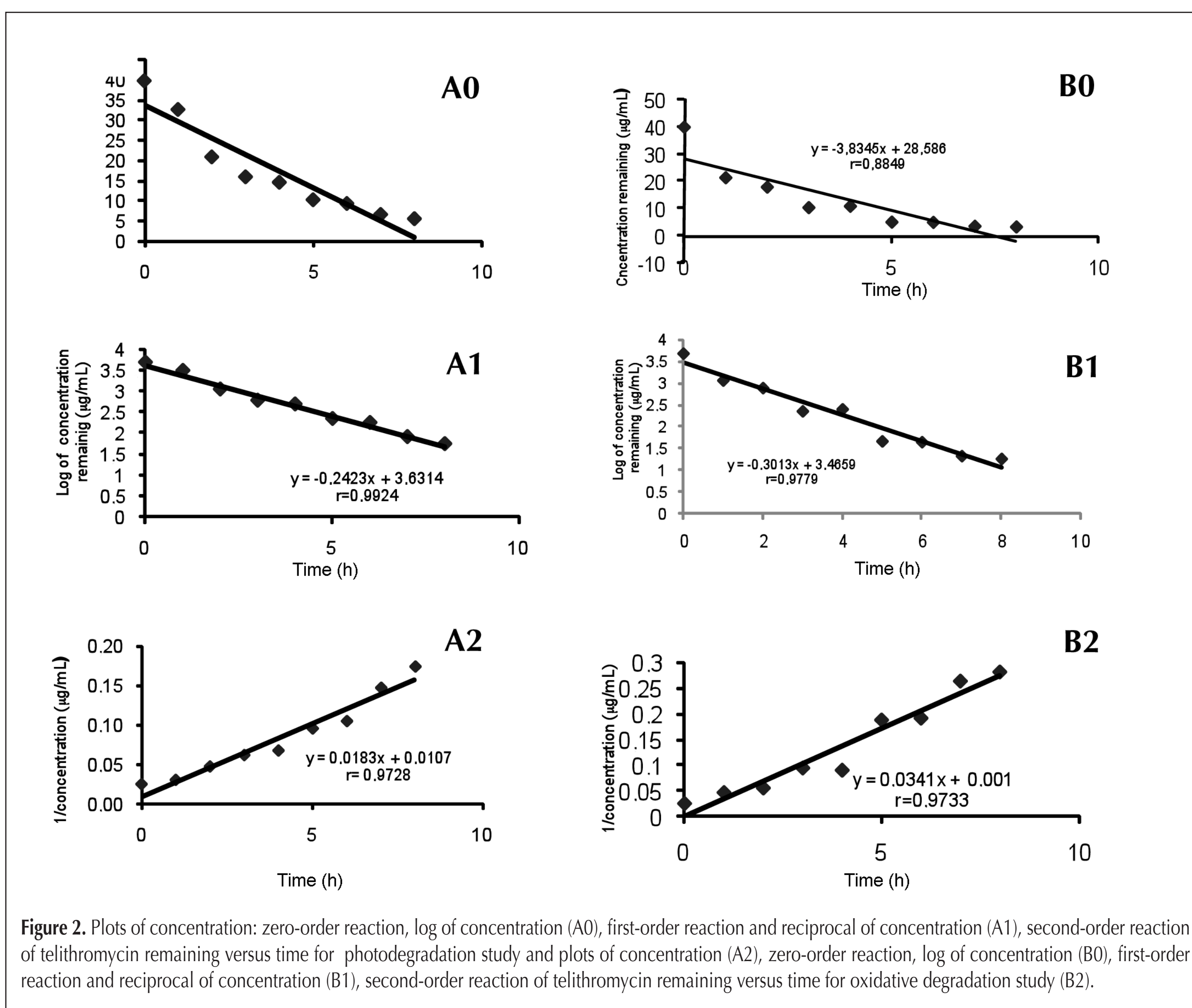

Figure 2. Plots of concentration: zero-order reaction, log of concentration (A0), first-order reaction and reciprocal of concentration (A1), second-order reaction of telithromycin remaining versus time for photodegradation study and plots of concentration (A2), zero-order reaction, log of concentration (B0), first-order reaction and reciprocal of concentration (B1), second-order reaction of telithromycin remaining versus time for oxidative degradation study (B2).

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$C = C_0 - kt$ $t_{90\%} = 0.1\ C_0/k$ (zero-order reaction)

$\ln C = \ln C_0 - kt$ $t_{90\%} = 0.106/k$ (first-order reaction)

$1/C = 1/C_0 + kt$ $t_{90\%} = 1/9k\ C_0$ (second-order reaction)

where C_0 is the concentration of the reactants under consideration at time zero, C is the concentration after reaction time t and k is the reaction rate constant.

Results

The effect of light and oxidation on the residual concentration of telithromycin in degradated samples are shown in Table I.

The kinetics of photodegradation and oxidative degradation were calculated through the fall in the drug concentration with the time. The concentration of the remaining telithromycin was calculated at each time interval for the three replicates, in comparison with the mean concentration of the standard solution of the drug in each degradation condition. The plots of concentration, log of concentration, and reciprocal of concentration of the drug remaining versus time are shown in Figure 2. Through the evaluation of the correlation coefficients, it can be demonstrated that the telithromycin solutions can be described by first-order kinetics under both experimental conditions used in this study. From the slopes of the straight lines, it was possible to calculate the first-order degradation rate constant k, and the t_{90} for each stress testing (Table II).

Discussion

The HPLC method was previously developed and validated (11) for the quantitation of telithromycin in pharmaceutical dosage forms. In this previous work, the preliminary stability study showed sensibility to oxidation and light. In this study, the HPLC method was applied in the determination of the kinetics of photodegradation and oxidation degradation of the telithromycin, and could effectively separate the drug from its degradation products, as shown in Figure 3. When the 1% hydrogen peroxide solution was used to promote the oxidative degradation, the results obtained did not shown significant degradation to determine the order of reaction. To determine the kinetics of degradation and to estimate kinetics parameters, it is essential to achieve at least 50% of degradation. When values are below 50%, it is not possible to set the order of reaction (14).

The purpose of the stability testing is to provide evidence

Table II. Degradation Rate Constant (k), and t_{90} for Telithromycin Solutions after Photodegradation and Oxidative Degradation Determined by the HPLC Method

	K (h^{-1})	t_{90} (h)
Photodegradation	0.2591	0.4091
Oxidative degradation	0.3951	0.2683

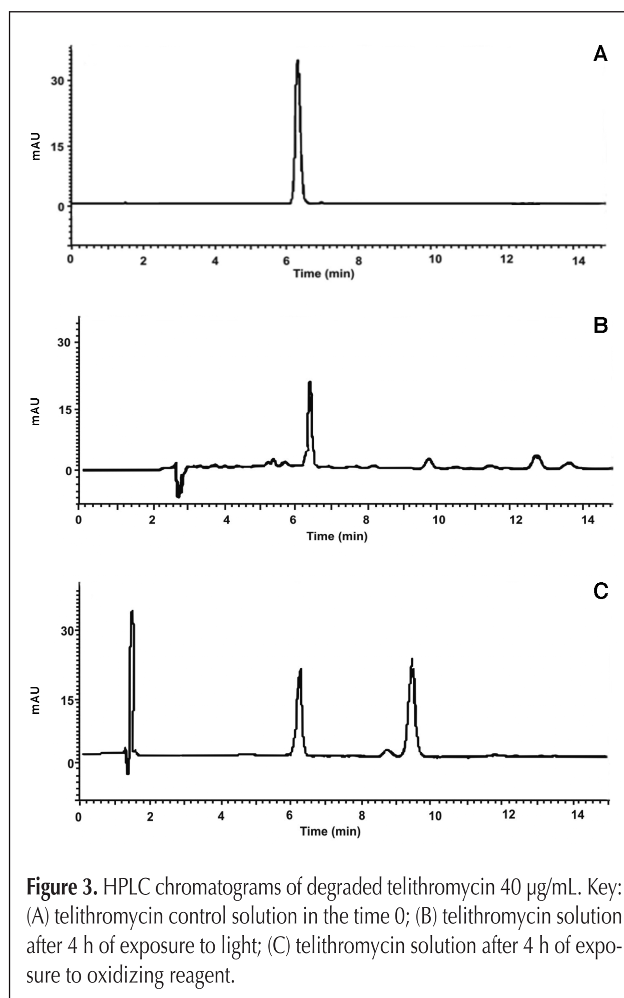

Figure 3. HPLC chromatograms of degraded telithromycin 40 µg/mL. Key: (A) telithromycin control solution in the time 0; (B) telithromycin solution after 4 h of exposure to light; (C) telithromycin solution after 4 h of exposure to oxidizing reagent.

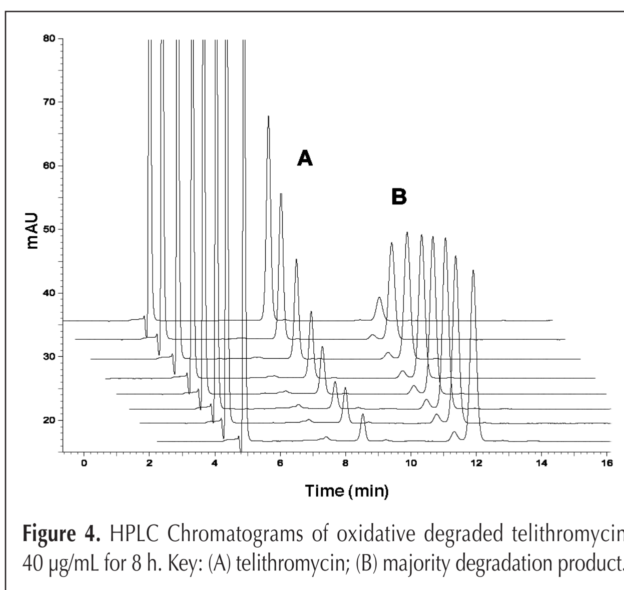

Figure 4. HPLC Chromatograms of oxidative degraded telithromycin 40 µg/mL for 8 h. Key: (A) telithromycin; (B) majority degradation product.

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regarding how the quality of the drug varies with time under the influence of light and oxidation. The stress testing is the first part of the stability evaluation and can help identify the likely degradation products, establish the degradation pathways, and the intrinsic stability of the molecule, as well as validate the stability indicating power of the analytical procedure used.

In this work, the kinetics of light and oxidative degradation of telithromycin were carried out through the employment of stress conditions. The exposure to light and oxidizing reagent were found to be important adverse stability-factors. The HPLC method was used for the determination of the drug in the degradated samples. The light and oxidative degradation profile of telithromycin were evaluated at different time intervals. After 4 h exposure to light, over 50% of the parent compound degraded. Typical chromatograms, showing the observed changes during the degradation in comparison to the initial sample, are demonstrated in Figure 3.

A majority degradation product at around 9.7 min was seen after the reaction between the telithromycin solution and 3% hydrogen peroxide solution. The peak around 1.5 min represents the 3% hydrogen peroxide solution. Figure 4 shows a decrease in the level of the drug and an increase of a majority degradation product during the time interval of this stress testing. No degradation products were found in the samples used as dark controls.

The chromatograms of the placebo solutions presented no peak; neither in the retention time of telithromycin, nor in the degradation products. Thus, there is no influence of the excipients in the determination of the kinetics of this drug. During the photodegradation condition, the temperature, which was controlled in the chamber, was always below 30°C.

Conclusions

The kinetics of photodegradation and oxidative degradation of the telithromycin solutions were determined. The photodegradation and oxidative degradation of telithromycin follow first-order reaction kinetics.

The stability-indicating HPLC method is able to separate telithromycin from its degradation products and tablets excipients for its sensitivity and reproductibility, after that it can be predicted the kinetics parameters of degradation rate constant, and t_{90}.

Acknowledgments

The authors thank CAPES and CNPQ (Brasilia, Brazil) for financial support.

References

1. *The Merck Index:* An encyclopedia of chemicals, drugs and biologicals, 13th Ed, Merck & Co., Inc., Whitehouse Station, NJ, 2001.
2. G. Ackermann and A.C. Rodloff. Drugs of the 21st century: telithromycin (HMR3647)—the first ketolides. *J. Antimicrob. Chemother.* **51:** 497–511 (2003).
3. T.C. Heninger, X. Xu, D. Abbanat, E.Z. Baum, B.D. Foleno, J.J. Hilliard, K. Bush, D.J. Hasta, and M.J. Macielag. Synthesis and antibacterial activity of C-6 carbamate ketolides, a novel series of orally active ketolides antibiotics. *J. Biorgan. Med. Chem.* **14:** 4495–4499 (2004).
4. A. Graul and J. Castañer. J. HMR–3647. *Drug of the Future* **23:** 591–597 (1998).
5. G.G. Zhanel, M. Walters, A. Noreddin, L.M. Vercaigne, A. Wierzbowski, J.M. Embil, A.S. Gin, S. Douthwaite, and D.J. Hoban. The ketolides a critical review. *Drugs* **62:** 1711–1804 (2002).
6. C. Perret, E. Weinling, D.H. Wessels, H.E. Scholtz, G. Montay, and E. Sultan. Pharmakocinetics and absolute oral bioavailability of an 800 mg oral dose of telithromycin in heathy young and elderly volunteers. *Chemotherapy* **48:** 217–223 (2002).
7. F. Traunmuller, R. Gattringer, A. Zeitlinger, W. Graninger, M. Muller, and C. Joukhadas. Determination of telithromycin in human plasma and microdialysates by high performance liquid chromatography. *J. Chromatogr. B* **822:** 133–136 (2005).
8. B. Lingerfelt and W.S. Champney. Macrolide and ketolide antibiotic separation by reversed phase high performance liquid chromatography. *J. Pharm. Biomed. Anal.* **20:** 459–469 (1999).
9. L.C. Vaucher, A.R. Breier, and E.S. Schapoval. Microbiological assay of telithromycin in tablets. *J. AOAC Int.* **89:** 1398–1402 (2006).
10. *The United States Pharmacopoeia,* 29th ed., United States Pharmacopoeial Convention, Rockville, MD, 2006, pp. 2644–2651.
11. L.C. Vaucher, C.S. Paim, A.D. Lange, and E.S. Schapoval. LC method for telithromycin in tablets: A stability-indicating assay. *Int. J. Pharm.* **366:** 82–87 (2009).
12. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology, Q2 (R1).
13. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Stability testing of new drugs substances and products.
14. N.E.S. Nudelman. Estabilidad de Medicamentos, El Ateneo, Buenos Aires, Argentina, 1975, p. 84.

Manuscript received March 16, 2009.
revision received June 22, 2009.