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Article

Validation of a Stability-Indicating RP-LC Method for the Determination of Tigecycline in Lyophilized Powder

Lucélia Magalhães da Silva* and Hérida Regina Nunes Salgado

Postgraduate Program in Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences of Araraquara- UNESP, Araraquara-SP, Brazil

*Author to whom correspondence should be addressed. Email: lucmsil@yahoo.com.br

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A reversed-phase liquid chromatography (RP-LC) method was validated for the determination of tigecycline in lyophilized powder. The LC method was conducted on a Luna C18 column (250 × 4.6 mm i.d.), maintained at room temperature. The mobile phase consisted of buffer containing sodium phosphate monobasic (0.015M) and oxalic acid (0.015M) (pH 7.0)–acetonitrile (75:25, v/v), run at a flow rate of 1.0 mL/min and using ultraviolet detection at 280 nm. The chromatographic separation was obtained with a retention time of 8.6 min, and was linear in the range of 40–100 μg/mL ($r^2 = 0.9997$). The specificity and stability-indicating capability of the method was proven through forced degradation studies, which also showed no interference of the excipients. The accuracy was 99.01% with a bias lower than 1.81%. The limits of detection and quantitation were 1.67 and 5.05 μg/mL, respectively. Moreover, method validation demonstrated satisfactory results for precision and robustness. The proposed method was applied for the analysis of the lyophilized powder formulation, contributing to improve the quality control and to assure the therapeutic efficacy.

Introduction

A disturbing global trend currently suggests the coming of a new post-antibiotic era, in which there are few antimicrobials available to treat new and emerging pathogens, fuelled by the use, overuse and misuse of antibiotic therapy (1). There is a need to develop new agents that overcome existing mechanisms of resistance displayed by multidrug-resistant bacteria (2). In this context, the development of new antimicrobials with activity against resistant pathogens and the study of analytical methodology to assure quality represent important clinical practice advances. Glycylcyclines, discovered in 1993, are structural analogues of tetracycline designed to avoid resistance mediated by efflux and ribosomal protection (3). Tigecycline, a novel, first-in-class glycylcycline, is a potent, broad-spectrum antibiotic that acts by inhibition of protein translation in bacteria (4). Tigecycline exhibits robust activity against bacterial isolates resistant to other antibiotic classes, including beta-lactams and fluoroquinolones, while resisting deactivation by most of the known tetracycline resistance mechanisms found in clinically significant bacteria (5). This antibiotic has been evaluated as monotherapy for serious infections in human clinical trials as a result of its microbiological, pharmacodynamic and pharmacokinetic properties (3, 6). Tigecycline is structurally derived from minocycline by adding a *tert*-butyl-glycylamido side chain to carbon 9 of the D ring of the tetracycline backbone (7). Figure 1 depicts the structural formulas of tigecycline (8). Chemically, tigecycline is (4S,4aS,5aR,12aS)-9-(2-tert-butylaminoacetylamino)-4,7-bis-dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide. Its chemical formula is $C_{29}H_{39}N_5O_8$, and its molecular weight is 585.65 Daltons (4, 9).

Few methods have been described to analyze tigecycline and its metabolites. Four high-performance liquid chromatography–ultraviolet (HPLC–UV) assays for the determination of tigecycline in biological materials have been found. The methods utilize C18 stationary phase and a combination of acetonitrile and buffer (ammonium acetate or phosphate), using both isocratic and gradient elution (10). The determination of tigecycline concentrations in Hank's balanced salts solution, human polymorphonuclear neutrophils and human serum was published by Li *et al.* (11). Another HPLC method was used by Bradford *et al.* (12) to determine whether the amount of oxygen dissolved in testing media is related to the variability of tigecycline minimum inhibitory concentrations (MICs). The safety, tolerability and pharmacokinetics of tigecycline were evaluated, assaying this drug in serum and urine by the HPLC method (13). The tigecycline metabolite profiling was performed by HPLC–UV method using two linear gradient eluted mobile phases. Additional HPLC conditions were used to characterize a polar metabolite that was not retained on the C18 columm. Separations were accomplished on a Waters Atlantis HILIC silica column using two mobile phases, trifluoracetic acid (TFA) in acetonitrile and TFA in water (4). In addition to HPLC–UV, HPLC–tandem mass spectrometry (MS-MS) methods have also been employed to determine tigecycline in biological fluids and tissues such as serum, urine, bronchoscopy and bronchoalveolar lavage, alveolar cells, gall bladder, bile, colon, bone, synovial fluid, lungs and rat bones (4, 13–16).

The development of stability-indicating assays using the approach of stress testing, as determined by the International Conference on Harmonization (ICH) guideline (17), is highly recommended for the quality control of pharmaceutical formulations, because the presence of impurities may influence the efficacy and safety of pharmaceuticals. Impurities and potential degradation products can change the chemical, pharmacological and toxicological properties of drugs, significantly impacting product activity and safety (18). Tigecycline is commercially available, but at the moment, there is no stability-indicating method published for the quantitative analysis of the drug in the presence of its degraded products. Additionally, most of the published analytical methods regarding the evaluation of tigecycline apply acid pH, which was this study determined to

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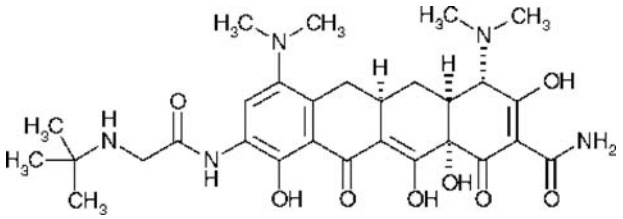

Figure 1. Chemical structure of tigecycline.

be detrimental to the separation of degradation products. Thus, the aim of the present article was to develop and validate a simple and fast stability-indicating reversed-phase liquid chromatography (RP-LC) method using a neutral pH for the quantitative analysis of tigecycline in lyophilized powder, and to evaluate the degraded products by mass spectrometry, assuring the therapeutic efficacy and contributing to improvement of the quality control.

Experimental

Chemical and reagents

The tigecycline reference substance was purchased from Sequoia Research Products (Oxford, UK). A total of four batches of Tygacil (Wyeth, New York, NY) lyophilized powder containing 50 mg of tigecycline were obtained from commercial sources within their shelf-life period, and were identified from 1 to 4. HPLC-grade acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ), oxalic acid from Synth (Diadema, Brazil) and sodium phosphate monobasic from Merck (Darmstadt, Germany). All chemicals were of pharmaceutical or special analytical grade. For all of the analyses, ultrapure water was purified using an Elix 3 coupled to a Milli-Q Gradient A10 system (Millipore, Bedford, MA).

Apparatus

The RP-LC method was performed on a Waters LC system (Waters Corporation, Milford, MA) equipped with a Waters 1525 binary pump, a Rheodyne Breeze 7725i manual injector and a Waters 2487 UV detector. The peak areas were integrated automatically by a computer using the Empower 2 software program.

The LC–MS experiments were performed on an ion trap mass spectrometer (Thermo Scientific, West Palm Beach, FL), model LCQ Fleet, equipped with an electrospray ionization (ESI) source in positive mode, set up in scan mode, using the Xcalibur V software program.

Preparation of reference substance solution

The stock solution was prepared by accurately weighing 5 mg of tigecycline reference substance, transferring to a 25-mL volumetric flask and diluting to volume with mobile phase buffer, obtaining a concentration of 200 μg/mL of tigecycline. The stock solution was daily prepared, diluted to an appropriate concentration in mobile phase buffer and filtered through a 0.45-μm membrane filter (Millipore).

Preparation of sample solutions

To prepare the sample solution, vials containing 50 mg of tigecycline were accurately weighed and mixed. An appropriate amount was transferred into an individual 25-mL volumetric flask and diluted to volume with mobile phase buffer. The final concentration of 200 μg/mL of the active pharmaceutical ingredient was daily prepared, diluted to an appropriate concentration with mobile phase buffer, filtered through a 0.45-μm membrane filter, and then injected, and the amount of the drug was calculated against the reference substance.

RP-LC method

The analysis of tigecyline in pharmaceutical formulations and degradation studies was conducted on a reversed-phase Phenomenex (Torrance, CA) Luna C18 column (250 × 4.6 mm i.d., with a particle size of 5 μm and pore size of 110 Å). A security guard holder (4.0 × 3.0 mm i.d.) was used to protect the analytical column. The LC system was operated isocratically at room temperature using a mobile phase consisting of buffer containing sodium phosphate monobasic (0.015M) and oxalic acid (0.015M) (pH 7.0)–acetonitrile (75:25, v/v). It was filtered through a 0.45-μm membrane filter (Millipore) and run at a flow rate of 1.0 mL/min, using UV detection at 280 nm. The injection volume was 20 μL for both the reference substance and the samples. Other mobile phases tested included: potassium phosphate 0.01M (pH 4.0)–acetonitrile (80:20 v/v); 0.01M potassium phosphate (pH 4.0) with 4 mM octasulfonic acid–acetonitrile (70:30 v/v); 0.03M potassium phosphate (pH 3.0)–acetonitrile (90:10 v/v); 0.03M sodium phosphate (pH 2.5)–acetonitrile (90:10 v/v); 0.03M sodium phosphate (pH 2.5)–methanol (80:20 v/v); 0.025M phosphoric acid (pH 3.0, adjusted with triethilamine)–acetonitrile (90:10 v/v).

LC–MS method

The LC–MS conditions were optimized with an injection volume of 20 μL of tigecycline reference solution (70 μg/mL) into the same chromatography column used in the RP-LC method. The LC system was operated isocratically at room temperature using a mobile phase consisting of acetonitrile–methanol–water–formic acid (12:8:80:0.05) with 4 mM ammonium acetate salt (14), run at flow rate of 0.5 mL/min, without splitter. The best response was obtained with electrospray capillary voltage of 2.0 V, capillary temperature of 275°C, sheath gas flow rate (N2) of 10 un and spray voltage of 5 kV. The mass spectrometry data were acquired in the mass-to-charge ratio (m/z) range between 100 and 750 atomic mass units (amu).

Validation of the RP-LC method

The method was validated using samples of pharmaceutical formulations with the label claim of 50 mg by determinations of the following parameters: specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness and system suitability test, following the ICH guidelines (17, 19).

Specificity

A stability-indicating method is defined as an analytical method that accurately quantifies the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities (20). The stability-indicating capability of the method was determined by subjecting a reference sample solution (200 μg/mL) to accelerated degradation by acidic, basic, neutral, oxidative and photolytic conditions to evaluate the interference in the quantitation of tigecycline (17). The acidic degradation was induced by preparing the sample solutions in 0.1M hydrochloric acid at 70°C for 5 h, and then cooling the solution and neutralizing with base. A sample solution prepared in 0.1M sodium hydroxide was used for basic hydrolysis evaluation, in which the solution was heated at 70°C for 5 h, cooled and neutralized with acid. For the study under neutral conditions, the drug was dissolved in water and heated at 70°C for 7 h. Oxidative degradation was induced by storing the sample solutions in 3% hydrogen peroxide at ambient temperature for 15 min, protected from light. Photodegradation was induced by exposing the samples in a photostability chamber to 200 W h/m^2 of near UV light for 24 h. After the procedures, the samples were diluted with the mobile phase buffer to a final concentration of 70 μg/mL. The interference of the excipients of the pharmaceutical formulation was determined by the injection of a sample containing only placebo (in-house solution of lyophilized powder excipient) and a sample containing placebo added to tigecyline at a concentration of 70 μg/mL.The stability-indicating capability of the method was then established by the acceptable separation of degradation product peaks from the tigecycline peak. Additionally, the tigecycline reference standard and degraded samples were analyzed by LC–MS.

Linearity and range

The linearity was determined by constructing three independent analytical curves in mobile phase buffer, each with seven reference substance concentrations of tigecyline, in the range of 40–100 μg/mL. Before injection of the solutions, the column was equilibrated for at least 20 min with the mobile phase flowing through the system. Three replicates of 20-μL injections of the reference solutions were made to verify the repeatability of the detector response. The peak areas of the chromatograms were plotted against the respective concentrations of tigecycline to obtain the analytical curve. The results were subjected to regression analysis by a least-squares method to calculate the calibration equation and determination coefficient.

Precision and accuracy

The precision of the method was determined by the repeatability and intermediate precision. Repeatability was examined by six evaluations of the same concentration sample of tigecycline on the same day and under the same experimental conditions. The intermediate precision of the method was assessed by conducting the analysis on three different days (inter-days), and also by other analysts performing the analysis in the same laboratory (between analysts). The accuracy was evaluated by applying the proposed method to the analysis of known amounts of the reference substance added to a sample solution containing 40 μg/mL of tigecycline, to obtain solutions at concentrations of 56, 70 and 84 μg/mL, equivalent to 80, 100 and 120% of the nominal analytical concentration, respectively. The accuracy was calculated as the percentage of the drug recovered from the sample and expressed as the percentage relative error (bias %) between the measured mean concentrations and added concentrations. Analysis of variance (ANOVA) and Student's *t*-test were performed to verify the absence of significant differences between the results of the accuracy and intermediate precision.

LOD and LOQ

The LOD and LOQ were calculated, as defined by ICH (19), using the mean values of three independent analytical curves, determined by a linear-regression model in which the factors 3.3 and 10 for the detection and quantitation limits, respectively, were multiplied by the ratio from the standard deviation of the intercept and the slope. The LOQ was also evaluated in an experimental assay.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. The robustness evaluation of the chromatographic method for tigecycline quantitation was performed using the method proposed by Youden and Steiner (21). Robustness was determined by analyzing the same samples (70 μg/mL) under variations of seven analytical parameters of the method, including different columns. Two of Youden's tests were conducted and the seven analytical parameters employed, as well as the introduced variations, are demonstrated in Table I. The analytical conditions at the nominal values are represented by capital letters and the conditions with small variations are represented by lowercase letters.

The results obtained of each experiment were represented by the letters *s*, *t*, *u*, *v*, *w*, *x*, *y* and *z*, respectively. From these results, it was possible to estimate the effect of each variable by obtaining the difference of the four analyses that have the nominal value (capital letter) and the four analyses with the alternative value (lowercase letter). For example, to evaluate the effect of the column supplier in the final result of the analyses, the following equation was employed: $[(s+t+u+v)/4 - (w+x+y+z)/4]$ (22). Considering the mean and standard deviation of the eight results, the following criterion was applied: If the value of the difference $(A-a \ldots G-g > s\sqrt{2})$, the variable has a significant effect and the method is sensitive to changes in the variable concerned (23).

System suitability test

A system suitability test was also conducted to evaluate the resolution and reproducibility of the system for the analysis to be performed, using seven replicate injections of a reference solution containing 70 μg/mL of tigecycline. The parameters measured were peak area, retention time, theoretical plates, capacity factor and tailing factor (peak symmetry).

Analysis of tigecycline in pharmaceutical formulations

For the quantitation of tigecycline in the lyophilized powder formulation, the respective stock solutions were diluted to an

Table I
Analytical Parameters, Values and Experimental Conditions during Robustness Evaluation of the Chromatographic Method

Analytical parameter	Value (*X*/*x*)	Experimental condition							
		1	2	3	4	5	6	7	8
Column supplier	*A*/*a* Test 1 (Phenomenex/ Waters) Test 2 (Phenomenex/ Waters)	*A*	*A*	*A*	*A*	*a*	*a*	*a*	*A*
Buffer pH	*B*/*b* Test 1 (7.0/7.5) Test 2 (7.0/6.5)	*B*	*B*	*b*	*b*	*B*	*B*	*b*	*B*
Mobile phase flow rate (mL/min)	*C*/*c* Test 1 (1.0/1.2) Test 2 (1.0/0.8)	*C*	*C*	*C*	*c*	*C*	*c*	*C*	*C*
Acetonitrile concentration (%)	*D*/*d* Test 1 (25/28) Test 2 (25/22)	*D*	*D*	*d*	*d*	*d*	*d*	*D*	*D*
Wavelength (nm)	*E*/*e* Test 1 (280/285) Test 2 (280/275)	*E*	*E*	*E*	*e*	*e*	*E*	*e*	*E*
Acetonitrile supplier	*F*/*f* Test 1 (J.T.Baker/Tedia) Test 2 (J.T.Baker/Tedia)	*F*	*F*	*f*	*F*	*F*	*f*	*f*	*F*
Buffer concentration (M)	*G*/*g* Test 1 (0.015/0.018) Test 2 (0.015/0.012)	*G*	*G*	*g*	*G*	*g*	*G*	*G*	*G*
Result		*s*	*t*	*u*	*v*	*w*	*x*	*y*	*z*

appropriate concentration (70 μg/mL) with mobile phase buffer, filtered and injected in triplicate, and the percentage recoveries of the drug were calculated against the reference substance.

Results and Discussion

Optimization of chromatographic conditions

Tigecycline is a structural analogue of tetracyclines, showing similar chemical properties to this group. Tetracyclines form chelate complexes with metal ions and adsorb on the silanol group in a reversed-phase column, so that tetracyclines are apt to appear as tailing peaks. To avoid their adsorption on RP columns, and to avoid forming chelate complexes, various methods have been reported, including RP column chromatography using mobile phases containing various acids (phosphoric, citric, tartaric and EDTA) and ion pair chromatography. However, tetracyclines have still shown extreme tailing on the RP-HPLC column, even when using mobile phases containing these acids, and only a mobile phase containing oxalic acid has resulted in no tailing peaks of the tetracyclines (24).

To obtain the best chromatographic conditions, different mobile phases were evaluated, and a mobile phase containing sodium phosphate, oxalic acid buffer (pH 7.0) and acetonitrile was selected because it provides the best chromatographic performance and acceptable peak characteristics, including tailing factor, number of theoretical plates and capacity factor. Moreover, suitable resolution of tigecycline and the degraded products was obtained, confirming the stability-indicating capability of the proposed method. The use of acetonitrile resulted in better selectivity, short analysis time and an improvement in the peak symmetry (approximately 1.15). The wavelength detection was evaluated in the range from 200 to 400 nm, and the wavelength of 280 nm was chosen due to its better sensitivity and signal-to-noise ratio.

The optimized conditions of the LC method were validated for the analysis of tigecycline in lyophilized powder formulation, due to the application for the quality control.

A typical chromatogram obtained by the proposed RP-LC method, demonstrating the resolution of the symmetrical peak corresponding to tigecycline, is shown in Figure 2. The retention time observed (8.6 min) allows a fast determination of the drug, free from any coeluting peak, which is suitable for quality control laboratories.

Method validation

The stability-indicating properties of the method are evaluated by forced degradation studies, particularly when no information is available about the potential degradation products. All of the conditions evaluated in the forced degradation studies resulted in the formation of degradation products. The oxidative condition exhibited significant and rapid decrease of the area, with six visible peaks and many other small peaks detected, confirming the high reactivity of the tigecycline with oxygen, made possible by the low stability of this drug. The neutral and basic conditions exhibited significant decreases of the area with four and five additional peaks detected, respectively. The degradation under the acidic condition was slower than neutral and basic conditions, with two additional peaks formed. Under the photolytic condition, a significant decrease of the area was also observed, with seven additional peaks detected (Figure 2). The tigecycline reference substance and degradation samples were also analyzed by MS. The tigecycline was susceptible to ESI in the positive mode, but some degraded products were not detected by MS because they were not ionized or their molecular weights were out of the lower mass range used in the study. MS spectra, as shown in Figure 3, resulted in the identification of three degraded products in the basic, acid, photolytic and neutral conditions, with m/z values of 117.2, 453.3 and 475.4, respectively. Under oxidative

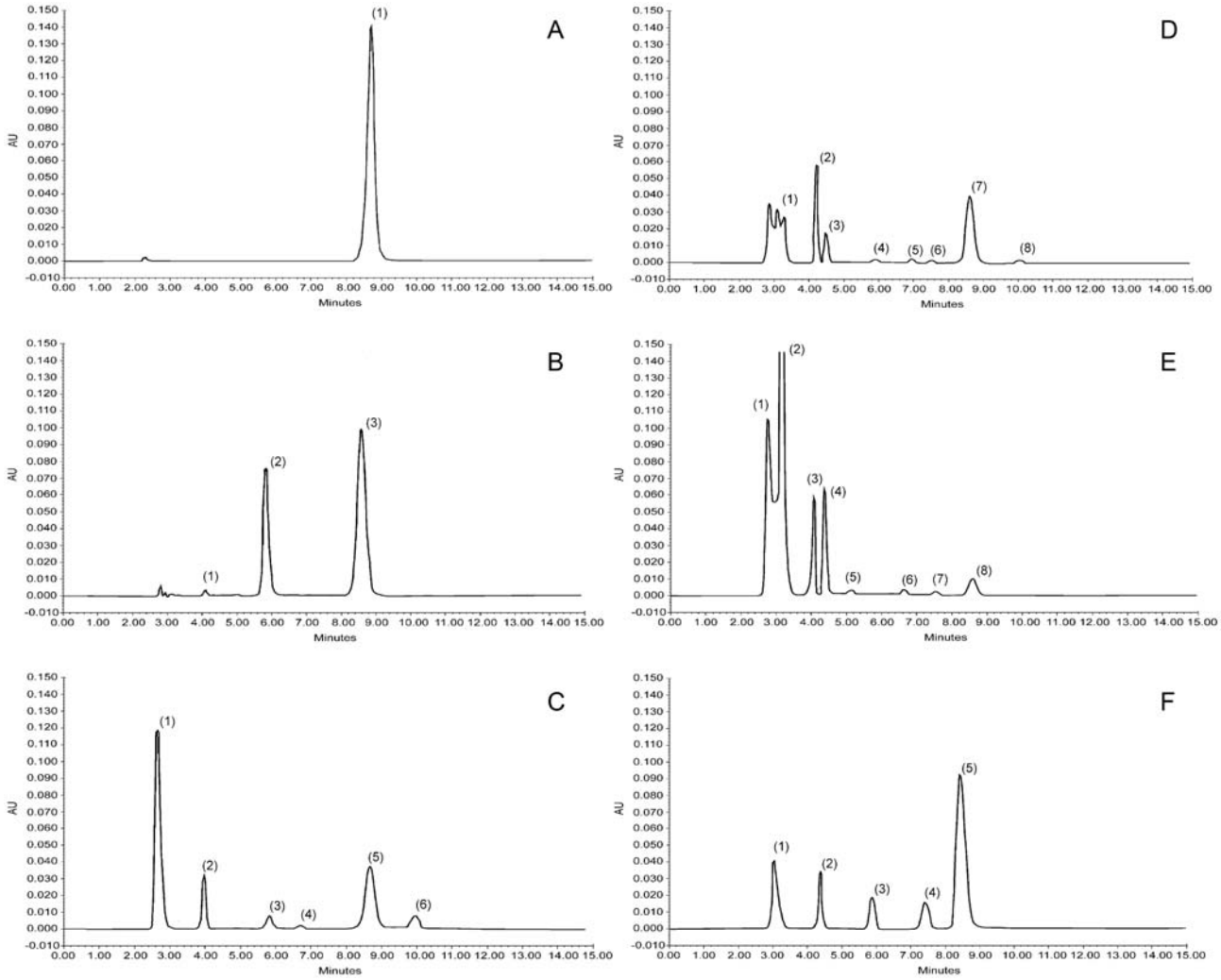

Figure 2. LC chromatograms of tigecycline (70 μg/mL): tigecycline reference substance solution, peak 1 = tigecycline (A); acid condition, peaks 1 and 2 = degraded forms, peak 3 = tigecycline (B); basic hydrolysis, peaks 1, 2, 3, 4 and 6 = degraded forms, peak 5 = tigecycline (C); exposure to UV light, peaks 1, 2, 3, 4, 5, 6 and 8 = degraded forms, peak 7 = tigecycline (D); oxidation, peaks 1, 3, 4, 5, 6 and 7 = degraded forms, peak 2 = hydrogen peroxide, peak 8 = tigecycline (E); neutral condition, peaks 1, 2, 3 and 4 = degraded forms, peak 5 = tigecycline (F).

conditions, four additional signals were observed, two of which were different from signals observed in the other conditions (130.2, 162.3, 453.3 and 475.4).

The relative standard deviation (RSD) values calculated in the system suitability test for the parameters tested were within the acceptable range (RSD < 2.0%), as shown in Table II, indicating that the system is suitable for the intended analysis.

The analytical curves constructed for tigecycline were found to be linear in the 40–100 μg/mL range. The value of the determination coefficient was calculated [$r^2 = 0.9997$, $y = (30773.77x \pm 393.79)x + (62280.15 \pm 15527.67)$, where x is concentration and y is the peak absolute area] indicated the linearity of the analytical curve for the method.

The precision, evaluated as the repeatability of the method, was studied by calculating the RSD for six determinations at 70 μg/mL, performed on the same day and under the same experimental conditions. The obtained RSD value was 1.12%. The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on three different days (inter-day); the mean values obtained were 110.72 and 123.24% with RSD 0.62 and 1.26%, respectively. The between-analyst precision was determined by calculating the mean values and the RSD for the analysis of two samples of the pharmaceutical formulation by two analysts; the values were found to be 110.91 and 122.98% with RSD 1.11 and 1.84%, respectively. The results are shown in Table III. The results of ANOVA and Student's *t*-test showed no variability with different days [F calculated (0.0090) < F critical (9.5521)] or different analysts [t calculated (0.0844) < T critical (4.3027)].

The accuracy was assessed from three replicate determinations of three different solutions containing 56, 70 and 84 μg/mL. The absolute means obtained for tigecycline are shown in Table IV, with a mean value of 99.01% and bias lower than 1.81%, demonstrating that the method is accurate within the desired range. Additionally, the results of the Student's t-test to

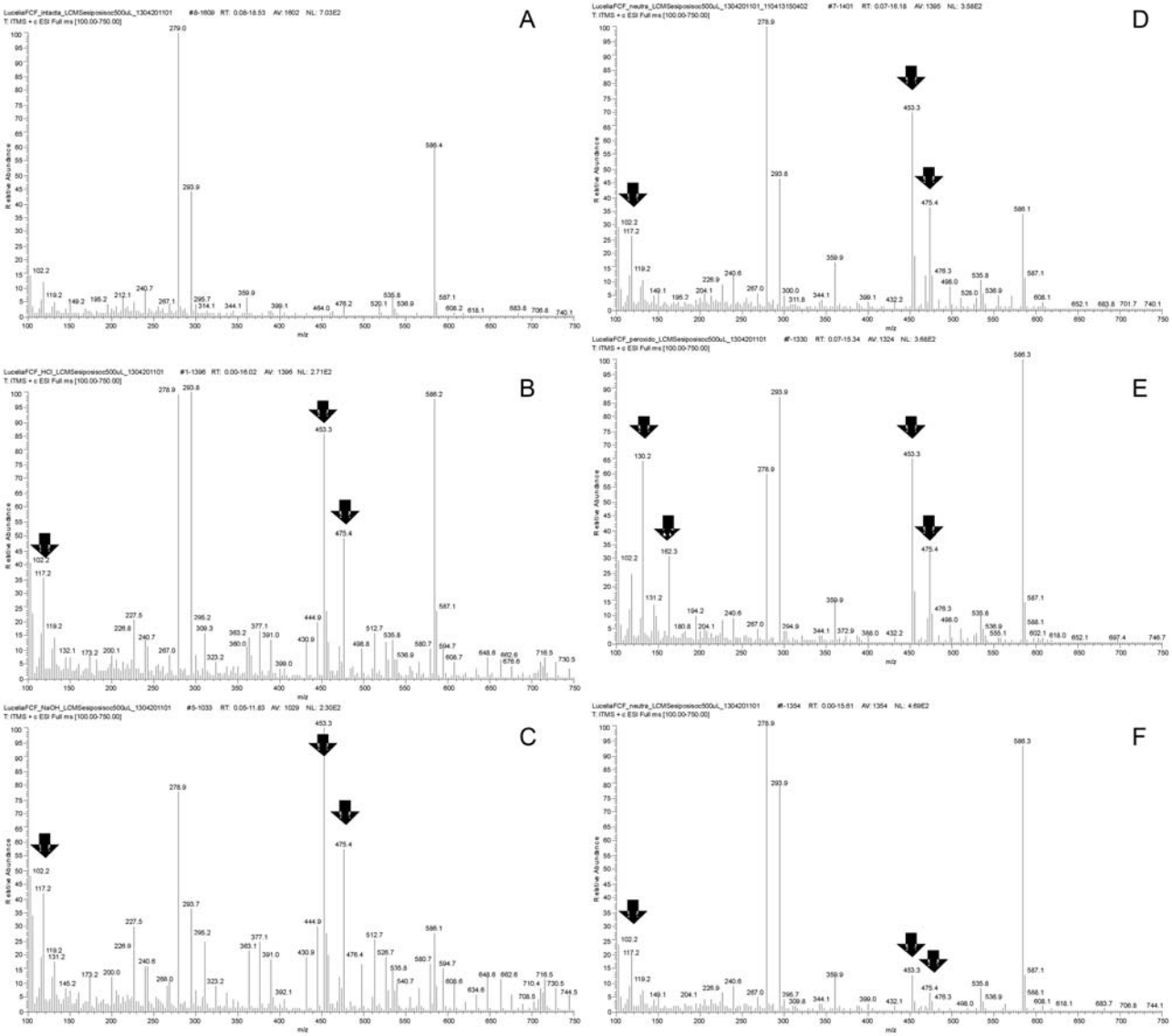

Figure 3. The full scan MS spectra of tigecycline: tigecycline reference substance solution (A); acid condition, degraded products = m/z 117.2, 453.3 and 475.4 (B); basic condition, degraded products = m/z 117.2, 453.3 and 475.4 (C); exposure to UV light, degraded products = m/z 117.2, 453.3 and 475.4 (D); oxidation, degraded products = m/z 130.2, 162.3, 453.3 and 475.4 (E); neutral condition, degraded products = m/z 117.2, 453.3 and 475.4 (F). Tigecycline = m/z 585.65.

Table II
Results of the System Suitability Test

	Tigecycline*			
Parameter	Minimum	Maximum	RSD (%)	Status
Theoretical plates	7,456.16	7,566.77	0.59	Passed
Retention time	8.65	8.72	0.31	Passed
Peak area	2,114,839	2,184,285	1.12	Passed
Tailing factor	1.12	1.16	1.42	Passed
Capacity factor	2.74	2.86	1.83	Passed

*Values from six replicates.

Table III
Inter-Day and Between-Analysts Precision Data of RP-LC for Tigecycline in Samples of Pharmaceutical Products

	Inter-day			Between-analysts		
Sample	Day	Concentration found* (%)	RSD (%)	Analysts	Concentration found* (%)	RSD (%)
1	1	110.04	0.62	A	110.04	1.11
	2	110.71		B	111.78	
	3	111.41				
2	1	123.61	1.26	A	124.58	1.85
	2	124.58		B	121.37	
	3	121.54				

*Mean of three replicates.

true added (16, 30 and 44 μg/mL) and found (Table IV) concentrations were [t calculated (3.0000) < T critical (4.3027)], [t calculated (0.0000) < T critical (4.3027)] and [t calculated (3.7500) < T critical (4.3027)], respectively, showing the accuracy of the method.

For calculating the LOD and LOQ, a calibration equation, $y = 30773.77x + 62280.15$, was generated by using the mean values of the three independent analytical curves. The LOD and LOQ were obtained by using the mean of the slope, $30773.77x \pm 393.79$, and the standard deviation of the intercept of the independent curves, determined by a linear regression line as 15527.67. The calculated LOD and LOQ were 1.66 and 5.05 μg/mL, respectively. The LOQ was evaluated in an experimental assay, with a precision lower than 5% and accuracy within ± 5%, and was found to be 2.5 μg/mL.

The results and the experimental range of the selected variables evaluated in the robustness assessment are given in Table V. The difference (X–x), mean values, standard deviations and criterions ($s\sqrt{2}$) were calculated and used to evaluate the results. The calculated criterions were 2.23 and 1.75 for Tests 1 and 2, respectively. The results meet the acceptance criterion, with no significant changes in the content results when the modifications were made in the experimental conditions, thus showing the method to be robust. The analysis performed by testing different columns resulted in changes in the retention time without effects on the determination of the drug in the pharmaceutical formulations.

Table IV
Accuracy of RP-LC for Tigecycline in Samples of Pharmaceutical Products

Added concentration (μg/mL)	Concentration found (μg/mL)	RSD (%)	Accuracy (%)	Bias* (%)
16	15.67	0.61	98.21	1.81
	15.82			
	15.64			
30	30.28	0.59	100.31	0.30
	30.08			
	29.92			
44	43.85	1.14	98.52	1.48
	42.86			
	43.34			

*Bias: [(Measured concentration – Nominal concentration)/Nominal concentration] × 100.

Method application

The proposed RP-LC method was applied for the determination of tigecycline in lyophilized powder, without prior separation of the excipient of the formulation, showing the applicability of the method for quality control analysis (Table VI). The results demonstrated elevated tigecycline content in the samples, however, these results are not considered to be irregular because official reference standards and specifications for tigecycline in lyophilized powder have not yet been established.

Conclusions

A novel, simple and sensitive method for the determination of tigecycline in lyophilized powder has been developed and validated. To our knowledge, this is the first LC method for the evaluation of tigecycline in pharmaceutical products. The results of the validation studies show that the RP-LC method is sensitive, accurate, specific and stability-indicating. The method possesses significant linearity ($r^2 = 0.9997$) and precision, with a mean RSD of 1.19% with no interference from the excipient or degradation products. The use of oxalic acid in the mobile phase was satisfactory to prevent the interaction of tigecyclines with the silanol groups of the stationary phase, thus avoiding the appearance of tailing peaks. Therefore, the proposed method was successfully applied and suggested for the routine analysis of tigecycline in pharmaceutical products, contributing to improvements in the quality control and assuring the therapeutic efficacy of this new drug.

Table VI
Determination of Tigecycline in Pharmaceutical Products by the RP-LC Method

Label claim		Experimental amount		
	Tigecycline	Tigecycline*		
Sample	Milligrams per vial	Milligrams	Recovery (%)	RSD (%)
1	50	57.31	114.62	1.37
2	50	56.57	113.14	0.94
3	50	59.77	119.54	1.22
4	50	59.46	118.91	0.18

*Mean of three replicates.

Table V
Effects of the Analytical Parameters on Content, during Robustness Testing of the Chromatographic Method for Tigecycline Quantitation

Youden's Test 1		Youden's Test 2	
Effect	Content* (%)	Effect	Content* (%)
Column supplier (A = Phenomenex; a = Waters)	109.35 – 108.38 = 0.98	Column supplier (A =Phenomenex; a = Waters)	110.74 – 111.77 = –1.03
Buffer pH (B = 7.0; b = 7.5)	108.67 – 109.06 = –0.39	Buffer pH (B = 7.0; b = 6.5)	111.23 – 111.28 = –0.05
Mobile phase flow rate (mL/min) (C = 1; c = 1.2)	108.86 – 108.87 = –0.01	Mobile phase flow rate (mL/min) (C = 1; c = 0.8)	110.97 – 111.53 = –0.56
Acetonitrile concentration (%) (D = 25; d = 28)	109.86 – 107.87 = 1.99	Acetonitrile concentration (%) (D = 25; d = 22)	110.70 – 111.81 = –1.11
Wavelength (nm) (E = 280; e = 285)	108.35 – 109.38 = –1.03	Wavelength (nm) (E = 280; e = 275)	111.32 – 111.19 = 0.13
Acetonitrile supplier (F = J.T. Baker; f = Tedia)	108.32 – 109.41 = –1.10	Acetonitrile supplier (F = J.T. Baker; f = Tedia)	111.73 – 110.77 = 0.96
Buffer concentration (M) (G = 0.015; g = 0.018)	109.46 – 108.27 = 1.19	Buffer concentration (M) (G = 0.015; g = 0.012)	110.58 – 111.93 = –1.35

* Mean of content obtained in normal conditions – Mean of content obtained in modified conditions; Reference values: 2.23 (Test 1) and 1.75 (Test 2).

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