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Development and validation of a stability-indication LC-UV method for determination of daptomycin injectable form and kinetic study in alkaline medium

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An isocratic liquid chromatography method (LC-UV) was developed and validated to determine daptomycin in injectable form. The method was carried out in a Waters XBridge C18 column (250 mm x 4.6 mm, 5µm). The mobile phase was composed of methanol/acetonitrile/buffer (pH 2.2) (40:30:30 v/v/v) at a flow rate of 1.0 mL^{-1}, using photodiode array (PDA) detection at 223 nm. The retention time obtained for daptomycin was 6.1 min and the method was linear in the range of 10 to 50 µg mL^{-1} (r= 0.9999). Forced degradation studies were performed to verify the specificity and stability-indicating capability of the method. The degradation kinetics under alkaline conditions was also evaluated. The method showed suitable accuracy (99.17%) and precision (RSD 0.59%) A two level full factorial design was used to determine method robustness. The proposed method was applied for the analysis of daptomycin injectable form, contributing to improve the quality control of this pharmaceutical product.

Introduction

The resistance of gram-positive pathogens such as staphylococci, streptococci and enterococci has been reported in several countries[1]. Since 2002, the Infectious Diseases Society of America (IDSA) has voiced concern with the lack of progress in developing new antimicrobial agents for the treatment of multi-resistant pathogens. In 2010 IDSA launched a campaign that aims to develop 10 new safe and effective antibiotics by 2020[2]. Among the newly developed antibiotics are linezolid, quinupristin-dalfopristin, tigecycline, telavancin and deftarolime fosamil[1,2].

Daptomycin (Figure 1, Cubicin®,Cubist Pharmaceuticals Inc.) is a new antibiotic, approved in 2003 by FDA. It is the first representing a new antibiotic class named cyclic peptides and is marketed only in injectable pharmaceutical form[3]. It was synthesized in 1980, produced from a strain of *Streptomyces roseosporus* and was originally named LY 146032[4,5].

It has bactericidal activity in vitro against most gram-positive aerobic and anaerobic pathogens of clinical importance such *Staphylococcus aureus,* including methicillin-resistant, *Streptococcus pyogenes* and *Enterococcus faecalis* but has no activity against gram-negative bacteria[1]. The daptomycin mechanism is different from any other approved antibiotic since it binds to the bacterial cell membrane causing its rapid depolarization due to potassium efflux which causes disruption of DNA, RNA and protein synthesis and fast bacterial death. This antibiotic is highly calcium dependent; therefore, it is not very active in the absence of calcium[6,7]. It is used for the treatment of several infections including vancomycin-resistant enterococcal infections, right-sided endocarditis with associated bacteremia, skin infections and other diseases caused by Gram-positive microorganisms[7,8].

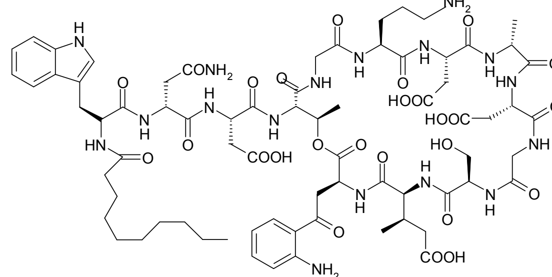

Figure 1 – Chemical structure of daptomycin.

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So far no methods to determine daptomycin in pharmaceutical form have been reported. In the literature we found some HPLC methods to determine daptomycin in biological fluids such as serum[9-11], plasma[12-16], and blood [6, 17]. In 2010 a method was also developed to determine daptomycin in blood, urine and peritoneal fluid[18].

This paper describes the first method aimed at assaying daptomycin injection. The method proposed was fully validated according to ICH guidelines[19] and it was also used to perform a degradation kinetics study of daptomycin in alkaline medium.

Experimental

Chemical and reagents

Daptomycin (DPT) reference substance was supplied by Hisun Pharmaceutical Co., Ltd. (Xukow Town, China). Daptomycin injection (Cubicin®, Novartis) was obtained at a local hospital, within its shelf life. HPLC-grade methanol and acetonitrile were obtained from Tedia (Farfield, Ohio, USA). All chemicals were of pharmaceutical or special analytic grade. Ultrapure water was purified byMega Purity System®.

Instrumentation and analytical conditions

The method was developed in a Shimadzu LC System (Kyoto, Japan) equipped with a CBM-20A system controller, SPD-M20A PDA detector, DGU-20A5 degasser and LC-20AT pump. The column used to perform the analysis was Waters XBridge C18 (250 mm x 4.6 mm, 5µ) maintained at 20 to 22°C. The mobile phase was methanol, acetonitrile and buffer pH 2.2 (40:30:30 v/v/v). The mobile phase was filtered through a 0.45 µm membrane filter (Milipore, Bedford, USA) and run at 1.0 mL min^{-1}; the detection was obtained at 223 nm. The peak area was integrated automatically by LC Solution Software Program and the quantitation was performed using the absolute peak area. The injection volume was 20 µL.

Solution preparation

Reference substance and sample solutions

The stock solutions of reference substance and samples were prepared by dissolving the equivalent of 10 mg of DPT in volumetric flasks with deionized water, in order to obtain a concentration of 100 µg mL^{-1}. The solutions were stored at 2-8°C, protected from light (maximum 7 days) and daily diluted to an appropriate concentration in mobile phase.

Buffer pH 2.2

The buffer was prepared by diluting 1.38 g of monobasic sodium phosphate in 1000 mL with deionized water and the pH was adjusted at 2.2 with phosphoric acid. The buffer was stored at 2-8°C and used during 30 days.

Method validation

The method was validated using samples of DPT injectable form according to ICH guideline (2005). The following parameters were evaluated: specificity, linearity, precision, accuracy and robustness.

Specificity

The ability to assess the analyte unequivocally in the presence of other components is known as specificity[19]. In order to verify this validation parameter, samples were subjected to forced degradation. The quantitation and purity of the DPT peak after forced degradation in alkaline, acidic and oxidative media, high temperature and exposure to UVA radiation were established. Peak purity index above 0.999 was considered acceptable[20]. Under all conditions previously described stock sample solution was used. Specific conditions are described below (n=3/condition).

Alkaline and acidic hydrolysis

To sample solution 5 mL of sodium hydroxide (NaOH) 0.01 M or 5 mL of hydrochloric acid (HCl) 0.1 M were added, to promote alkaline and acid hydrolysis, respectively. Alkaline solutions were maintained at room temperature during 30 minutes and neutralized with HCl 0.1 M. Acidic solutions were maintained at room temperature during 24 hours and neutralized with NaOH 1 M. The entire content was transferred to a 20 mL volumetric flask and volume was completed with mobile phase obtaining a concentration of 25 µg mL^{-1}.

Oxidative condition

Five milliliters of hydrogen peroxide H_2O_2 30% were added to the sample solution and stored protected from light, at room temperature, for 10 hours. The entire content was diluted in a 20 mL volumetric flask and diluted with mobile phase obtaining a concentration of 25 µg mL^{-1}.

Photodegradation

One milliliter of sample solution (25 µg mL^{-1}) was exposed to UVA radiation (352 nm) for 12 hours in a covered transparent container in a light chamber. Containers with sample solutions protected from light were also placed in the chamber in order to verify any interference due to temperature rise. Samples were analyzed immediately after the end of exposure.

Thermal degradation

Sample solution at 25 µg mL^{-1} was placed in a sealed glass bottle and placed in a drying oven at 40 ºC for 6 hours. After this period, aliquots of the solutions were analyzed.

Linearity

The determination of method linearity was evaluated through the construction of threeanalytical curves, each one with five concentrations of DPT in the range of 10 to 50 µg mL^{-1} (10, 20, 30,

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40 and 50 µg mL^{-1}).Analysis of variance was used to perform statistical evaluation analysis.

Precision

The determination of precision was assessed by repeatability (intra-day) and intermediate precision, expressed as relative standard deviation (RSD). The intra-day precision was performed by determination of six samples on the same day prepared by the same analyst. Intermediate precision was verified by analyzing six sample solutions prepared independently, changing day and analyst. Allsample solutions were prepared as previously described, at 25 µg mL^{-1}.

Accuracy

Accuracy was determined by adding known amounts of DPT reference substance to samples at the beginning of the process in three levels (70, 100 and 130% of work concentration). To obtain these solutions, 2 mL ofsample solution (100 µg mL^{-1}) were placed in 20 mL volumetric flasks to which 1.5, 3.0 or 4.5 mL of reference sample (100 µg mL^{-1}) were added. Dilutions were made in mobile phase to achieve final concentrations of 17.5, 25.0 and 32.5 µg mL^{-1} respectively. This procedure was performed in triplicate. Solutions of sample (10 µg ml^{-1}) and reference substance (10 µg ml^{-1}) were also prepared and analyzed.

Robustness

Robustness evaluates potential sources of variation in one or more method responses. Factors to be considered involve analytical procedure and environmental conditions. They may be qualitative, quantitative or a mixture of both. Some of the factors that can be investigated for an HPLC method include mobile phase pH, flow rate, column temperature, amount of organic modifier, column manufacturer, wavelength and others[21]. In order to assess the robustness of the proposed method, a full factorial design (2^3) with two levels (low and high) and three factors was carried out. Three relevant factors from these experiments were chosen and the percentage of methanol in mobile phase, flow and pH of mobile phase, name x1, x2 and x3, respectively were investigated (Table 1). A sample solution at 25 µg mL^{-1} was used to measure the effect of factors and the selected experiments were performed randomly as shown in Table 3.

Table 1 – Factors and levels investigated in full factorial design

Factor	Level		
	-1	0	1
x1: MeOH concentration (%)	36	40	44
x2: flow	0.9	1.0	1.1
x3: pH	1.9	2.2	2.5

Stability standard solution

To determine the stability of daptomycin standard solution, the stock solution tested was maintained at 2-8°C for 7 days. During this period aliquots of this solution were diluted to the work concentration and analyzed, in order to evaluate changes in amount when compared with freshly prepared solutions.

System suitability

The system suitability test was determined to confirm that the equipment was suitable for the analyses to be performed.In order to verify it, chromatographic parameters and RSD values obtained fromthe injection of six replicates of the reference solution 25µg mL^{-1} were determined.

Kinetic studies

To determine the degradation kinetics in alkaline medium, to 5 mL of DPT solution at 100 µg mL^{-1}5 mL of 0.01 M sodium hydroxide solution were added. At times 0, 10, 30, 60, 90, 120 and 180 minutes, the solutions were neutralized with 0.1 M hydrochloric acid and the volume was made up 20 mL with mobile phase.

Degradation kinetics was determined by the decrease in drug concentration over time, calculated by the graphic method. Zero order, first order and second order graphics were drawnby plotting drug residual content *versus* time, ln of drug residual content *versus* time and 1/drug residual content *versus* time, respectively. The correlation coefficient was calculated and the best fit was considered to establish the kinetic order[22]. The half-life ($t_{1/2}$) was determined from the k-value, being $t_{1/2} = ln\ 2/k$. The t_{90} (time for 10% decomposition) were obtained by equation inherent to the reaction order. The kinetic degradation was evaluated in triplicate each time.

Statistical software

General data were evaluated by analysis of variance at 5% of significance level. The statistical analyses of robustness were performed by Minitab®v. 16 software (Minitab Inc., State College, PA, USA).

Results and discussion

Method optimization

In order to develop a method that would meet the recommended analytical parameters with a suitable retention time, several preliminary tests were performed. Due to the presence of two distinct pka's in molecule, 4.0 and 10.0, acidic and alkaline respectively[23] we decided to work with low pH values, to prevent ionization of acid groups of molecule. Without the using of acetonitrile in the mobile phase, daptomycin was detected after two hours. Thus, it was included on its composition, which resulted in shorter analysis time. As aqueous phase, we at first used a solution of 0.1% formic acid (pH 2.7), employed in several methods used to determine daptomycin in biological fluids [14,16,18]. However, the combination of formic acid, acetonitrile and methanol did not allow

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a good separation of daptomycin and its degradation products. Changing formic acid for phosphate buffer pH 2.2, specificity and suitable peak symmetry were obtained. Detection wavelength was determined by an UV-scan of a 25 µg mL^{-1} daptomycin reference substance solution. To avoid the accumulation of substances strongly retained on the column it was washed with acetonitrile after about ten injections of the sample or standard solution[13].

Thus, daptomycin assay was obtained with the following conditions: acetonitrile, methanol and phosphate buffer pH 2.2 (30:40:30 v/v/v), flow rate of 1.0 mL min^{-1}, detection at 223 nm, Waters XBridgeC18 column (250 mm x 4.6 mm, 5µm) maintained at 20 to 22°C. With these conditions, the retention time of DPT 6.1 minutes and suitable values of resolution, tailing factor and theoretical plates were obtained. A typical chromatogram obtaining by this method is shown in Figure 2.

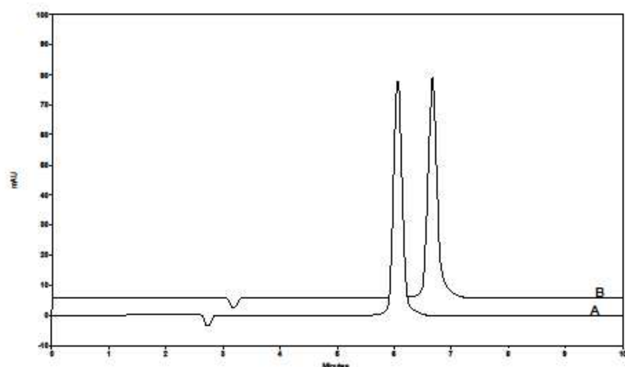

Figure 2 – Chromatograms of daptomycin reference substance (A) and daptomycin injection (B)

Method validation

Specificity

Since a proactive approach to developing a stability indicative HPLC method should involve forced degradation at the early stages of development[24], we started the validation procedures by stress testing. DPT was not degraded in acid medium or by thermal degradation under the conditions tested, the residual content being near 100% after reaction. Degradation of DPT in acidic media was reported by Kirsch et al.[25], by use of temperature (60 ºC) and acidic pH values, for extended time period. Those conditions induced to generation of anhydrodaptomycin and β-asp daptomycin isomer. In the conditions employed by us, no degradation was observed.

After 12 hours of UVA radiation exposure the DPT content was near 46% (Figure 3-B). The drug was degraded in oxidative and alkaline media, the residual content being around 74% and 85% in these conditions, respectively (Figure 3-C and 3-D). From these experiments, additional peaks were observed in chromatograms, probably from degradations products, but in alkaline conditions their presence is more evident. Muangsiri and Kirsch[26] reported the degradation pathway of daptomycin under alkaline conditions and suggested that degradation occurred either by hydrolytic cleavage of the ester bond between the C-terminal amino acid residue and the threonine side chain of the fourth amino acid residue or by the hydrolytic cleavage of an amide bond. PDA detector was used to determine the peak purity index. Under all conditions, the values were higher than 0.999, which confirms the method specificity.

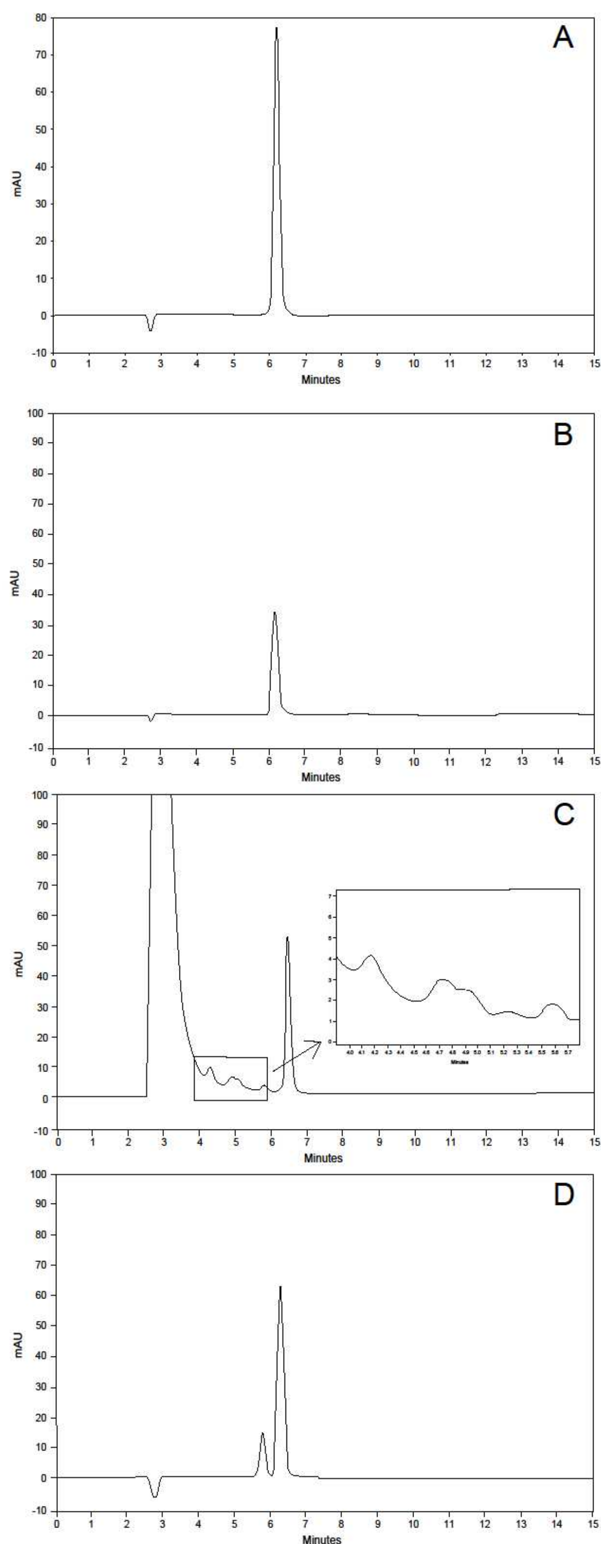

Figure 3 – LC chromatograms of daptomycin (25µg mL^{-1}). (A) reference substance of daptomycin; (B) UVA radiation; (C) oxidative medium and (D) alkaline medium

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Linearity

The analytical curve for daptomycin was considered linear in the range of 10-50 µg mL^{-1} with a correlation coefficient of 0.9999 and linear equation y=34987x - 4590.7, where x is the concentration and y is the absolute peak area (mAU). The validity was checked by analysis of variance, which indicated linear regression ($p < 0.05$) and no significant deviation from linearity ($p > 0.05$).

Precision

The method precision was evaluated by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as relative standard deviation (RSD). The intra-day RSD values were 0.69% (100.17%, n=6, analyst 1, day 1) and 0.53% (100.29%, n=6, analyst 2, day 2). The inter-day RSD value was 0.59% (100.23%, n=12). The RSD results are in agreement with recommended value, indicating precision of the method proposed[27].

Accuracy

The accuracy was verified by recovery test, through three determinations of three independent samples solutions containing different levels of DPT around the usual concentration of the method. The mean recovery obtained was 99.17%, with RSD 1.25%.The results are shown in Table 2 and indicate the accuracy of the method proposed.

Table 2 - Results of daptomycin reference standard recovery in the injectable daptomycin samples

Level (%)	Concentration added (µg mL^{-1})	Concentration recovered (µg mL^{-1})	Recovery (%)	Mean (%)	RSD (%)
		7.59	101.24		
70	7.5	7.44	99.24	100.14	1.01
		7.49	99.94		
		15.05	100.34		
100	15.0	14.73	98.17	99.27	1.09
		14.89	99.29		
		22.31	99.11		
130	22.5	21.95	97.55	98.09	0.89
		21.97	97.63		
			Mean and RSD	99.17	1.25

Robustness

Robustness can be described as the capacity to reproduce the analytical method under different circumstances without unexpected differences occurring in the results obtained[21].It is important to use a factorial analysis on method robustness because of the possibility of evaluatingthe influence of all experimental variables of interest and the interaction effects on the analytical responses [28]. By robustness evaluation it is possible to determinewhich sources of variation must be more tightly controlled during the execution of the method.

We conducted 2^3 full factorial design and in this design second and third order interaction effects can be determined. The three factors chosen to evaluate the robustness method were methanol concentration (x1), flow (x2) and pH mobile phase (x3); second order interactions can be evaluated by x1x2, x1x3, x2x3 and x1x2x3 represent third order interaction. The effects of method modifications were evaluated in three analytical responses: peak asymmetry, theoretical plates and assay. The results are shown in Table 3.

Table 3 - Full factorial design of three factors with optimized condition points and respective responses

Sample	Random order	Factors			Responses		
		x1	x2	x3	Peak symmetry	Theoretical plates	Assay (%)
1	10	1	-1	-1	1.06	8684.80	99.35
2	5	-1	1	1	1.14	7163.23	101.11
3	11	1	1	-1	1.18	7240.40	101.34
4	6	-1	-1	-1	1.14	8343.05	100.18
5	4	-1	-1	1	1.05	8689.23	101.45
6	9	1	1	1	1.08	7516.61	99.52
7	7	-1	1	-1	1.19	7102.64	99.54
8	8	1	-1	1	1.05	8733.75	101.69
9	1	0	0	0	1.12	7806.95	100.36
10	2	0	0	0	1.13	7822.83	101.24
11	3	0	0	0	1.12	7848.96	100.65

The significance of the factors and interaction of the factors over the assay value was evaluated by ANOVA and Pareto chart of the standardized effects. The *P*-values obtained by ANOVA ranged from 0.072 to 0.793 (corresponding to interaction of the three evaluated factors and methanol percentual, respectively). The Pareto graph (Figure 4) consists of bars, whose length is proportional to the absolute value of the estimated effect divided by the pseudo standard error.The codes A, B and C correspond to the analytical factors evaluated. When an interaction between two factors occurs, this is indicated by a bar with their combination. The chart includes a vertical line at the critical t-value for α of 0.05. Effects that cross the line are considered significant for the factor or factors combination analyzed. From these result, it was concluded that no single factor or their combination (second or third order) produced responses outside acceptable limits for the method, which indicated that the method is robust for the determination of DPT in injectable form.

Stability of standard solution

Standard solution stability was evaluated by comparing the results of the standard solution stored at 2-8°C with those obtained for the

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standard prepared on the same day. The RSD values during the 7 days were < 2.0%, the DPT assay being 98.7%, on day 7.

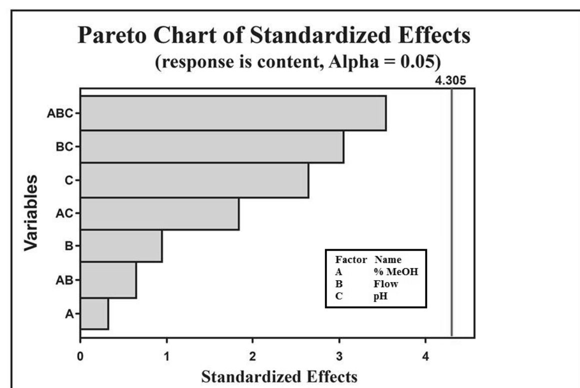

Figure 4 – Pareto Chart of Standardized Effects

System suitability

System suitability was evaluated by six replicate injections of DPT reference solution (25 µg mL^{-1}). The chromatographic parameters obtained were: theoretical plates, 7801 (RSD 1.36%); tailing factor, 1.19 (RSD 1.81%); capacity factor, 5.06 (RSD 0.55%). The RSD values among areas of six injections and the retention time were 1.12% and 0.41%, respectively. All these values were in accordance with the recommended ones[29].

Kinetic studies

Zero order, first order and second order models were used for modeling the kinetics of daptomycin degradation. The correlation coefficient was calculated and the best correlation coefficient was considered to establish the kinetic order. The degradation of DPT follows first order kinetics, since there is a linear relationship between the inverse of the residual concentration versus time (r = 0.9953) [22]. The rate constant order (k) was determined by the graphic equation and the value obtained was 0.0047ug mL.min^{-1}.

The time obtained through equation $t90\% = 0.106/k$ is 22.55 minutes, very close to the value obtained experimentally (16% of degradation in 30 minutes). The time required for DPT to be reduced to half the original value (half-life) was evaluated by $t_{1/2} = 2{,}303/k \log Co/C$ equation and time obtained was 130.81 minutes, in the conditions used.

Conclusions

The method proposed proved to be simple, linear, precise, accurate, specific and robust in range from 10 to 50µg mL^{-1}, meeting the ICH requirements. Considering the results, the method can be applied to the routine quality control of the injectable form and in stability studies; it also represents a contribution to improve the quality of this pharmaceutical product.

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Notes and references

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1. D.A.Enoch, J.M. Bygott, M.L Daly and J.A. Karas, *J Infect*, 2007, **55**, 205-213.
2. H.W. Boucher, G.H. Talbot, D.K. Benjamin Jr., J. Bradley, R.J. Guidos, R.N. Jones, B.E. Murray, R.A.Bonomo and D. Gilbert, *Clin Infect Dis,* 2013, **56**, 1685-1694.
3. R. Sauermann, M. Rothenburger, W. Graninger and C. Joukhadar, *Pharmacology*, 2008, **81**, 79-91.
4. G.M. Eliopoulos, S. Willey, E.Reiszner, P. G. Spitzer, G. Caputo and R.C. Moellering Jr, *Antimicrob Agents Chemother*, 1986, 30, **4**,53-535.
5. M. Debono, M. Barnhart, C.B. Carrell, J.A. Hoffmann, J.L. Occolowitz, B.J. Abbott, D.S. Fukuda and R.L Hamill, *J Antibiot*, 1987, 40, **6**, 761-777.
6. E Gikas, F.N. Bazoti, P. Fanourgiakis, E. Perivolioti, A. Roussidis, A. Skoutelis and A. Tsarbopoulos, *Biomed Chromatogr*, 2009, 24, 522-527.
7. L. M. Koeth, G., M. Thorne, *Clin Microbiol Newsl*, 2010, **32**, 161-169.
8. H.S. Sader, G.J. Moet, D.J. Farrell and R. N. Jones, *Diagn Microbiol Infect Dis*, 2011, **70**, 412-416.
9. M. J. Rybak, E. M. Bailey, k. C. Lamp and G. W. Kaatz, *Antimicrob Agents Chemother*,1992, **36**, 5, 1109-1114.
10. C.A. Deryke, C. Sutherland, B. Zhang, D.P. Nicolau and J.L. Kuti, *Antimicrob Agents Chemother*, 2006, **50**, 11, 3529-3534.
11. C. M. Tobin, J. M. Darville, A. M. Lovering and A. P. MacGowan, *J Antimicrob Chemother*, 2008, **62**, 1462-1476.
12. B. H. Dvorchik, D. Brazier, M. F. DeBruin and R. D. Arbeit, *Antimicrob Agents Chemother*, 2003, **47**, 13-18.
13. J. Martens-Lobenhofer, J. T. Kielstein, C. Oye and S.M. Bode-Boger, *J Chromatogr B*, 2008, **875**, 546-550.
14. L. Baietto, A, D´Avolio, F. G. de Rosa, S. Garazzino, m. Michelazzo, G. Ventimiglia, M. Siccardi, M. Simiele, M. Sciandra and G. Di Perri, *Anal Bioanal Chem*, 2010,**396**, 791-798.
15. F.N. Bazoti, E. Gikas, A. Skoutelis and A. Tsarbopoulos. *J Pharm Biomed Anal*. 2011, **56**, 78-85.
16. M.C Verdier, D. Bentué-Ferrer, O. Tribut, N. Collet, M. Revest and E. Bellissant.*Clin Chem Lab Med*, 2011,**49**, 69-75.
17. P. Olszowy, M. Szultka, P. Fuchs, R. Kegler, R. Mundkowski, W. Miekisch, J. Schubert and B. Buszewski. *J Pharm Biomed Anal*, 2010, **53**, 1022-1027.
18. H. G. Gika, F. Michopoulos, D. Divanis, S. Metalidis, P. Nikolaidis and G.A. Theodoridis. *Anal Bioanal Chem*, 2010, **397**, 2191-2197.
19. ICH – 2005. Guideline on Validation of Analytical Procedures: Text and Methodology.
20. D.G. Watson, Edinburgh: Elsevier Churchill Livingstone, 2005.
21.Y. V. Heyden, A. Nijhuis, J. Smeyers-Verbeke, B.G.M Vandeginste and D.L. Massart. *J Pharm Biomed Anal*, 2001, **24**, 723-753.

Analytical Methods Accepted Manuscript

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22. S. Silambarasan, J. Abraham. *J Taiwan Institute Chem Engineers*, 2013, **44**, 438–445.
23. Scifinder Scholar. American Chemical Society (ACS), 2012.
24. K. M. Alsante, A. Ando, R. Brown, J. Ensing, T.D. Hatajik, W. Kong and Y. Tsuda. *Adv Drug Deliv Rev*, 2007, **59**, 29-37.
25. L. E. Kirsch, R. M. Molloy, M. Debono, P. Baker, K. Z. Farid. *Pharm Res*, 1989, **6**, 387-393.
26.W. Muangsiri, L. Kirsch. *J Pharm Sci*, 2001, **90**, 1066-1075.
27. M. Bakshi,S. Singh. *J Pharm Biomed Anal,* 2002, **28**, 1011-1040.
28. R. F. Teofilo, M.M.C Ferreira, *Quim Nova*, 2006, **29**, 338-350.
29. CDER – 1994. Validation of Chromatographic Methods.

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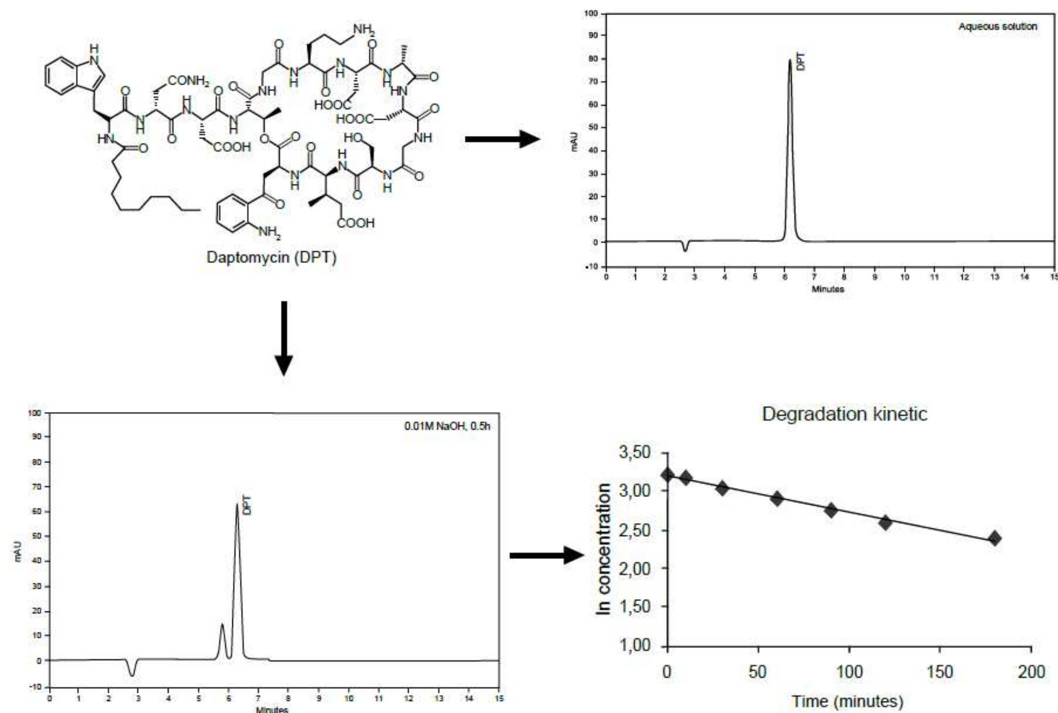
Graphical and textual abstract

Manuscript: Development and validation of a stability-indication LC-UV method for determination of daptomycin injectable and kinetic study in alkaline medium

In this study was developed ahigh-pressure liquid chromatographic method in order to quantify daptomycin in injectable form. The method was validated in accordance with the ICH requirements showing specificity, linearity, precision accuracy and robustness, which was evaluated by 2-Level 2^3 factorial design. Also was evaluated the kinetic study of daptomycin in alkaline medium. The proposed HPLC method was successfully applied for the quantitative analysis of daptomycin pharmaceutical form and stability studies.



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