Article

BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902019000318367

Evaluation of the stability of Polymyxin B in saline and glucose solutions using LC-MS/MS

Juliana Kich Castilhos[1], Thaís Luise Dillenburg[1], Marina Venzon Antunes[2], Letícia Scribel[3], Alexandre Prehn Zavascki[4], Rafael Linden[2], Simone Gasparin Verza[1]*

[1]Bioanalysis Laboratory, Institute of Health Sciences, Feevale University, Novo Hamburgo-RS, Brazil, [2]Toxicological Analysis Laboratory, Institute of Health Sciences, Feevale University, Novo Hamburgo-RS, Brazil, [3]Federal Institute of Education, Science and Technology of Rio Grande do Sul – Campus Porto Alegre, Brazil, [4]Department of Internal Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

---

Polymyxins are a group of antibacterial substances and have remained the drugs of choice for treatment of resistant Gram-negative bacilli. Polymyxin B is administered by intravenous infusion and requires the reconstitution of lyophilized powder with 0.9% saline or 5% glucose solutions. To date, there is little information about polymyxin stability in different infusions solutions, especially at 40 ºC, a temperature that is recommended to study drug stability as it accelerates degradation reactions. Therefore, in this work an analytical method using LC-MS/MS was developed, validated and applied to determine the stability of polymyxin B diluted in 0.9% saline or 5% glucose solutions at 25 ºC and 40 ºC. The stability of polymyxin B solutions was evaluated during 72 hours. Polymyxin B1 and B2 were stable for 24 hours in saline (0.9%) and glucose solution (5%), however a significant degradation of polymyxin B1 and B2 was observed after 48 hours and 72 hours of assay. The reduction of polymyxin content was evidenced in both saline and glucose media, at room temperature as well as at 40 °C. No significant differences in pH of polymyxin solutions (glucose or saline) were evidenced during stability assay.

Keywords: Polymyxin. Stability. LC-MS/MS. Glucose solutions. Saline solutions.

INTRODUCTION

Polymyxins are a group of antibacterial substances developed for clinical use in 1947. Released in the late 1950s, they remained largely unexplored due to their toxicity and the availability of other more tolerable groups of antibiotics including β-lactams and aminoglycosides (Zavascki *et al.,* 2007). However, polymyxins has re-emerged in clinical practice in the last 15 years owing to the worldwide dissemination of extensively-drug-resistant Gram-negative infections, notably, *Psedomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumonia* (Pogue, Ortwine, Kaye, 2017).

Polymyxin B, a member of the class of polymyxins, consists mainly of a mixture of polymyxins B1 and B2, which are usually derived from *Bacillus polymyxa* and administered as sulfate salts (USP, 2015). The polymyxin B package insert indicates that the intravenous administration of polymyxin B requires the reconstitution of the lyophilized powder (500,000 IU) with sterile water for injection and the infusion of this mixture must be made in 300 to 500 mL solutions of either 0.9% saline or 5% glucose (Trissel, 2012). However, such a volume may be excessively high for some critically ill patients who have either risk of developing fluid overload states or have restriction of fluid infusion due to several conditions. Therefore, stability of polymyxin B in lower volume solutions is of paramount importance and for which the administration should be performed slowly (Lim *et al.*, 2016).

Previous studies reported stability of polymyxins B1, E1 and E2 subjected to different pH values and temperatures (Orwa *et al.*, 2002). The stability of polymyxin B was evaluated also in 0.9% saline solutions stored at room temperature and under refrigeration using a microbiological assay (He *et al.*, 2010). In a more recent study the stability of polymyxin B was evaluated in various reduced infusion volumes of 0.045%, 0.225% and 0.9% saline, and 5% dextrose stored at 4, 25 and 30 °C (Lim *et al.*, 2016).

***Correspondence:** S. G. Verza. Instituto de Ciências da Saúde, Universidade Feevale, ERS 239, nº 2755, Novo Hamburgo, RS, Brazil, Postal code 93525-075. Phone: +55 51 3586 8800. E-mail: simonev@feevale.br

Nonetheless, stability studies of polymyxin B in 0.9% saline and 5% glucose solutions at room temperature and 40 °C is unavailable in scientific literature, especially using high-performance analytical methods such as LC-MS/MS. Therefore, the objective of this study was to evaluate the stability of polymyxins B1 and B2 in 0.9% saline and 5% glucose infusion solutions, at room temperature and at 40 °C for 72 hours. It is worth noting the recommendation of the temperature of 40 °C to study drug stability, as it accelerates degradation reactions providing indication of long-term stability (ICH 2003; WHO 2009). For drug quantification, we employed an LC-MS/MS methodology previously developed and validated.

MATERIAL AND METHODS

Standard, samples and other materials

The reference substance of polymyxin B sulfate (batch #BCBF8382V) was purchased from Sigma-Aldrich® (Missouri, EUA), and the assay samples were purchased from Eurofarma® (Brazil). Saline solutions (0.9%) and 5% glucose solutions (Baxter®, Brazil), as well as sterile water for injection (Isofarma®, Brazil) were also purchased. The analyses were performed using 0.1% formic acid (Merck®, Germany), methanol (Merck®, Germany) and purified water obtained using a Milli-Q® system (Merck Millipore Corporation®, France). For solvent filtration, a 0.22 µm Sartorius Stedim Biotech (Germany) membrane was used.

Equipment and analytical conditions

The quantification of polymyxin B was performed on a liquid chromatograph with mass detector (LC-MS/MS) from Thermo Scientific™ (United States), model UltiMate™ 3000 with RS column housing, XRS autosampler, and XRS pump all from Thermo Scientific. The software Thermo Xcalibur Roadmap was used for data management.

During the analyses, an ACE 3 C18 column (50 mm × 2.1 mm id; 3 µm) was used, conditioned at a temperature of 30 °C. The mobile phase consisted of 0.1% formic acid (A) and methanol (B) at flow rate of 0.3 mL/min in linear gradient system: 0 to 5.0 min, 10 to 18% B; 5.0 to 5.5 min, 18% B; 5.5 to 7.5 min, 18 to 95% B; 7.5 to 8.0 min, 95% B; and 8.0 to 11.0 min, 95 to 10% B. The volume of sample injection was 10 µL.

Detection was performed using positive mode electrospray ionization, and the predominant ions produced were $[M + 2]^{2+}$ monitoring m/z transitions 602.5 for polymyxin B1 and 595.5 for polymyxin B2. The parameters optimized to carry out the analyzes were capillary voltage of 5.0 kV, capillary temperature of 200 °C, vaporizer temperature of 432 ºC, cone gas flow of 15 Arb and nitrogen nebulizer gas flow of 50 Arb.

Validation of the analytical method

The analytical method was validated considering specificity, linearity, accuracy, precision (ICH, 2005) and the suitability of the analytical method was assured.

The specificity of the analytical method was evaluated by injecting 0.9% saline and 5% glucose solutions under the analytical conditions described above. Linearity was assessed based on three analytical curves prepared on three consecutive days in the concentration range of 5 to 30 µg/mL. For this purpose, a 5mg polymyxin standard solution was prepared 10 mL in a volumetric flask using acetonitrile in water (20:80 v/v) (LiChrosolv®, Germany). From this solution, further dilutions were made using a solution of 0.1% formic acid and methanol (80:20 v/v), comprising the concentration range aforementioned. The limits of detection (LOD) and quantification (LOQ) were calculated from the analytical curve (ICH, 2005). Accuracy was determined by recovery assay. For that purpose, solutions of 7.5 µg/mL, 17.5 µg/mL and 27.5 µg/mL polymyxin were prepared in 0.9% saline and 5% glucose solutions. The content recovered was calculated in percentage. Intra-assay precision was evaluated by 9 analyses of the mean point of the analytical curve (20 µg/mL) and the inter-day precision by means of the analysis of the results obtained at low, medium and high concentrations obtained for three different analytical curves prepared on three consecutive days. Both results were expressed as relative standard deviation (RSD%) (ICH, 2005).

Stability analysis

Polymyxin B samples submitted to the stability assay were individually prepared by diluting one ampoule of polymyxin B (500,000 IU), which is equivalent to 50 mg, in 2 mL of sterile water for injection. Subsequently these samples were injected into 500 mL infusion bags containing 0.9% saline or 500 mL infusion bags containing 5% glucose, and maintained at room temperature (25 °C) or in a heating oven at 40 °C (De Leo®, DU215CD , Brazil). All samples were obtained in triplicates.

For the LC-MS/MS analyses, all samples were diluted to 20 µg/mL with 0.1% formic acid in methanol

(80:20 v/v). The analyses were performed at the assay times of 0, 4, 8, 24, 48 and 72 hours and the percentage of polymyxin B1 and B2 was expressed. The pH of saline and glucose solutions of polymyxin B1 and B2 was measured at the same time interval mentioned above.

Statistical analysis

The influence of time and temperature on the stability of polymyxins B1 was analyzed by one-way analysis of variance (ANOVA), and subsequently, by a Tukey test using SPSS version 22 for windows. A value of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

LC-MS/MS analyses

The analytical method was proposed based on the chromatographic conditions described previously (Thomas *et al.,* 2012) with modifications. In the analytical conditions presented here, there was co-elution of the polymyxin B1 and B2 signals around 7.7-7.9 min. The detection was not influenced by the co-elution since it was performed monitoring m/z transitions: m/z 593.7 → m/z 482.20 and m/z 586.7 → m/z 101.00 for polymyxin B1 and B2, respectively (Figure 1).

The quantification of polymyxin B1 and B2 was satisfactory based on the validation parameters (Table I). The calibration curves of polymyxin B1 and B2 were found to be linear, both with correlation coefficients (r^2) > 0.994 in the concentration range of 5.0-30 µg/mL. The intra-day and inter-day precision tests were considered satisfactory with coefficients of variation (RSD) lower than 2.93% and 4.08%, respectively. The method's accuracy was within range of 96.1% to 104.1% for polymyxin B1 and 97.9% to 103.0 % for polymyxin B2.

Analyzing the results obtained during the stability tests, polymyxin B1 and B2 were stable for 24 hours when it was diluted in saline (0.9%) and glucose solution (5%).

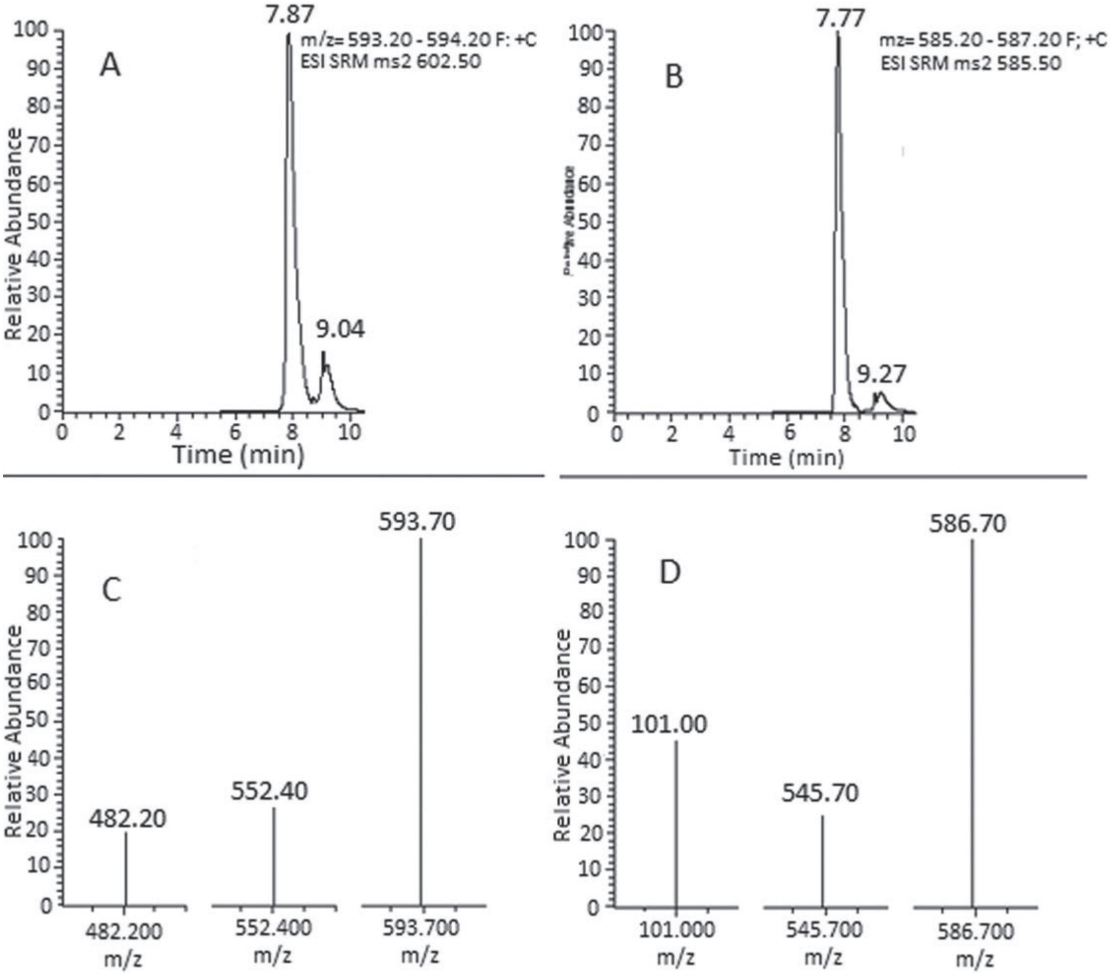

FIGURE 1 - Chromatograms of standard solutions: Polymyxin B1 (A) and Polymyxin B2 (B). Mass transitions from Polymyxin B1 (C) and Polymyxin B2 (D).

TABLE I - Results of analytical method validation

Validation parameter		Results	
		Polymyxin B1	**Polymyxin B2**
Equation		y = 4931352 x – 1,9E+07	y = 3450874x – 1,1E+07
r^2		0.9960	0.9948
LOD[a] (µg/mL)		0.183	0.209
LOQ[b] (µg/mL)		1.611	1.699
Intra-day precision (RSD[c]%) (n = 9)		≤ 2.42%	≤ 2.93%
Inter-day precision (RSD[c]%) (n = 9)		≤ 4.08%	≤ 3.96%
Accuracy			
5% Glucose	7.5 µg/mL	99.20% (± 0.02)	102.61 (± 1.96)
	17.5 µg/mL	97.67% (± 3.33)	98.37 (± 3.59)
	27.5 µg/mL	104.08% (± 2.87)	100.72 (± 1.93)
0,9% Saline	7.5 µg/mL	102.77% (± 2.80)	103.01 (± 0.94)
	17.5 µg/mL	96.09% (± 1.62)	102.77 (± 2.65)
	27.5 µg/mL	97.39% (± 3.09)	97.88 (± 2.71)

[a]LOD, limit of detection; [b]LOQ, limit of quantification ; [c]RSD%, relative standard deviation

TABLE II - Percentage of Polymyxin B1 and B2 during the stability assay

			Polymyxin B1				
Diluent	**Temperature**	**0**	**4 hours**	**8 hours**	**24 hours**	**48 hours**	**72 hours**
0.9% Saline	Room temperature	100[a]	99.0[a] ± 2.2	101.2[a] ± 4.8	96.8[a] ± 1.7	73.9[b] ± 6.7	81.2[b] ± 4.0
	40 °C	100[a]	100.8[a] ± 3.3	100.7[a] ± 3.0	98.1[a] ± 1.6	81.9[b] ± 4.2	86.6[b] ± 0.9
5% Glucose	Room temperature	100[a]	104.8[a] ± 3.4	96.9[a] ± 4.9	93.5[a] ± 2.0	73.3[b] ± 5.1	79.1[b] ± 2.5
	40 °C	100[a]	104.3[a] ± 7.1	100.8[a] ± 2.9	86.3[b] ± 1.9	81.7[b] ± 4.0	83.3[b] ± 2.4
			Polymyxin B2				
Diluent	**Temperature**	**0**	**4 hours**	**8 hours**	**24 hours**	**48 hours**	**72 hours**
0.9% Saline	Room temperature	100[a]	98.9[a] ± 3.4	100.4[a] ± 4.0	92.5[a] ± 1.2	80.2[b] ± 3.6	87.1[b] ± 4.4
	40 °C	100[a]	100.8[a] ± 3.6	100.6[a] ± 2.2	103.3[a] ± 1.6	88.4[b] ± 5.1	87.8[b] ± 3.0
5% Glucose	Room temperature	100[a]	102.9[a] ± 6.8	95.8[a] ± 3.6	93.2[a] ± 2.9	82.0[b] ± 4.7	85.7[b] ± 5.6
	40 °C	100[a]	106.9[a] ± 5.4	100.4[a] ± 3.9	90.7[a] ± 2.3	90.7[b] ± 4.2	86.9[b] ± 1.1

[ab] Averages in the same line followed by the same character do not differ significantly ($p>0.05$) based on Tukey test

For all samples, a reduction of polymyxin B concentration greater than 5% was observed after 24 hours. Meantime, no significant difference between the contents of polymyxin B1 and B2 during the first 24h was evidenced.

In order to verify the influence of temperature and time at stability of polymyxin B1 and B2 one-way analysis of variance (ANOVA), followed by Tukey test was used. The analysis of variance revealed a significant difference between the groups. A significant degradation of polymyxin B1 and B2 was observed after 48 hours, a reduction that continued up to the 72 hours of assay. The reduction of polymyxin content was evidenced in both saline and glucose media, at room temperature as well as at 40 °C. Temperature and media (saline solution or glucose solution) did not appear to have a significant influence on the stability of diluted polymyxin B.

The results obtained in this work are similar to those reported by Lim *et al.* (2016) who determined that polymyxin B was stable in high concentrations in different types of infusion solutions for at least 24 hours at temperatures of 25 and 30 °C. At 4 °C, polymyxin B remained stable for up to 168 hours. It is interesting to note that analyses were not performed at 40 °C as in the present study. The temperature of 40 °C is suggested for international guidelines to provide information's during accelerated storage conditions (WHO, 2009). If significant changes occur during accelerated stability studies a discussion should be provided to address the effect of it on product storage conditions. In our work polymyxin B was diluted in glucose or saline solutions and the temperature of 40 °C didn't promoted significant changes in polymyxin B content when compared with 25 °C.

He *et al.* 2010 evaluated the stability of polymyxin B under refrigeration (4 °C) and room temperature (25 °C) by microbiological assay. As in the present work, the authors observed that in the first 24 hours the concentration of polymyxin remained close to 95% at both temperatures, demonstrating the stability of polymyxin B. In accordance with our work, He *et al.* (2010) observed that after 48 hours, polymyxin B degraded almost 10%, and after 72 hours its concentration was below 90%. Additionally, the authors reported that at the end of 168 hours, polymyxin B concentrations were 75.4% at 25 °C and 78.9% at 4 °C, respectively.

Physical-chemical instabilities can be evidenced by pH changes of testing solutions. United States Pharmacopoeia specification for 5% dextrose injection is pH 3.2–6.5 (USP, 2015). The pH measured for the 0.9% sodium chloride injection in our study was within the range of 5.4 to 5.8, and for glucose solution (5%) the pH range was 4.2 to 4.7. No statistical differences were evidenced in these pH values during 72 hs. The results obtained in this work are in agreement with reported by Lim *et al.* (2016) who evidenced no statistical differences in pH and osmolarity values of polymyxin B solutions over time.

In previous studies He *et al.* 2010 evidenced a shorter stability of polymyxin B diluted in 0.9 % sodium chloride solutions, when compared with polymyxin diluted in 0.5% glucose solutions. The authors observed that the pH of 0.9 % sodium chloride solutions was less acidic than 5% dextrose solutions and mentioned that differences in pH can influence polymyxin B stability, however these results require further investigation, according to the authors.

Orwa *et al.* (2002) evaluated the stability of polymyxins B1, E1 and E2 in aqueous solutions submitted to different pH values and temperatures. The authors concluded that polymyxins B1, E1 and E2 are more susceptible to degradation at neutral or basic pH values.

The stability of polymyxins has also been evaluated in different analytical conditions over the years. Taylor *et al.* (1994) evaluated the chemical stability of polymyxin B in aqueous sodium phosphate monobasic buffer at different temperatures (32 °C to 52 °C) and at different pH values (2.0 to 10.3) over a period of 30 days. The authors showed that a pH below 7.0 has little effect on the stability of polymyxin B, but pH values above 7.0 promote drug decomposition reactions.

CONCLUSIONS

In this work we proposed and validated an analytical method that can be applied to stability studies of polymyxin B. The present work evidenced that polymyxins B1 and B2 diluted in 5% glucose solution and 0.9 % saline solution remain stable for 24 hours and undergo degradation after this time. Based on the results obtained in this study, it is possible to support the safety of the use of polymyxin B in saline (0.9%) and glucose solutions (5%) in an infusion course of 24 hours. No statistical differences were observed in pH values of glucose solutions and saline solutions containing polymyxin B and no differences were evidenced, with respect stability of polymyxin B, when it was diluted in saline or glucose solutions at 25ºC and 40ºC. Considering this, in our analysis conditions, the choice of any of these dissolutions media would be appropriate, considering the polymyxin B stability.

DISCLOSURE OF INTEREST

The authors report no conflict of interest.

REFERENCES

He J, Figueroa DA, Lim T-P, Chow DS, Tam VH. Stability of polymyxin B sulfate diluted in 0.9% sodium chloride injection and stored at 4 or 25 C. Am J Heal Pharm. 2010;67(14):1191-4.

ICH. Harmonised Tripartite Guideline. Stability Testing of New Drug Substances and Products Q1A(R2). 2003.

ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1) Guideline on Validation of Analytical Procedures: Methodology developed to complement the Parent Guideline. 2005.

Lim T-P, Hee DK-H, Lee W, Teo JQ-M, Cai Y, Chia SY-H, et al. Physicochemical Stability Study of Polymyxin B in Various Infusion Solutions for Administration to Critically Ill Patients. Ann Pharmacother. 2016;50(9):790-2.

Orwa J, Govaerts C, Gevers K, Roets E, Van Schepdael A, Hoogmartens J. Study of the stability of polymyxins B1, E1 and E2 in aqueous solution using liquid chromatography and mass spectrometry. J Pharm Biomed Anal. 2002;29(1-2):203-12.

Pogue JM, Ortwine JK, Kaye KS. Clinical considerations for optimal use of the polymyxins: A focus on agent selection and dosing. Clin Microbiol Infect. 2017;23(4):229-33.

Taylor RB, Richards RME, Low AS, Hardie L. Chemical stability of polymyxin B in aqueous solution. Int J Pharm. 1994;102(1-3):201-6.

Thomas TA, Broun EC, Abildskov KM, Kubin CJ, Horan J, Yin MT, et al. High performance liquid chromatographyg-mass spectrometry assay for polymyxin B1 and B2 in human plasma. Ther Drug Monit. 2012;34(4):398-405.

Trissel LA. Handbook of injectable drugs. 17th ed. Bethesda, Maryland: American Society of Health-System Pharmacists; 2012.

United States Pharmacopeial Convention. The United States pharmacopeia : the national formulary. Maryland: Rockville; 2015.

WHO. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO Tech Rep Ser. 2009;(953):87-130.

Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: A critical review. J Antimicrob Chemother. 2007;60(6):1206-15.

Received for publication on 09th May 2018
Accepted for publication on 23rd September 2018