Original Article

Influence of Concentration and Temperature on Stability of Imipenem Focused on Solutions for Extended Infusion

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Abstract

Background: Imipenem remains active against most Gram-positive and Gram-negative organisms. This study aimed to evaluate chemical stability of imipenem in 2 commonly used concentrations when stored in 3 various temperatures.

Methods: Imipenem injection powder was used to prepare 5 mL and 10 mg/mL of imipenem in .9% sodium chloride solution. Prepared solutions in PVC bags were stored at 25°C, 30°C, and 40°C. The solutions were investigated over 0, 1, 2, 3, 4, and 6 hours using HPLC analysis. The association between drug stability, temperature, and concentration was determined.

Results: The 5 mg/mL solutions of brand A and B imipenem mL were stable for 6 hours at 25°C, 30°C, and 40°C, respectively. For 10 mg/mL, the solution of brand A was stable for 3 hours and brand B was stable for 6 hours at 25°C. Also, brand A and B imipenem solutions at the concentration of 10 mg/mL were stable for less than 1 hour at 30°C and 40°C.

Conclusion: The stability of imipenem injection solution was affected by temperature and concentration. Increasing in temperature and drug concentration resulted in decreased stability of imipenem. Suitable temperature and drug concentration should be concerned when this drug is given by extended infusion.

Keywords

stability, imipenem, extended infusion, temperature, concentration

Introduction

The prevalence of multidrug-resistant (MDR) Gram-negative bacterial infection is increasing in several regions worldwide and associated with high mortality rates due to limited alternative drugs and gradual development of new antimicrobial agents.[1,2] Imipenem is 1 of antimicrobial agents that is still active against gram-negative and gram-positive pathogens, including the infection of MDR gram-negative bacteria. This drug is also a commonly used antibiotic in critically ill patients.3 The most important pharmacokinetic/pharmacodynamic (PK/PD) parameter correlating with therapeutic efficacy of imipenem is the percentage of time above the minimum inhibitory concentration (MIC)—the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism—(%T > MIC), so extended or continuous infusion of imipenem is a crucial factor of success in using imipenem.[4]

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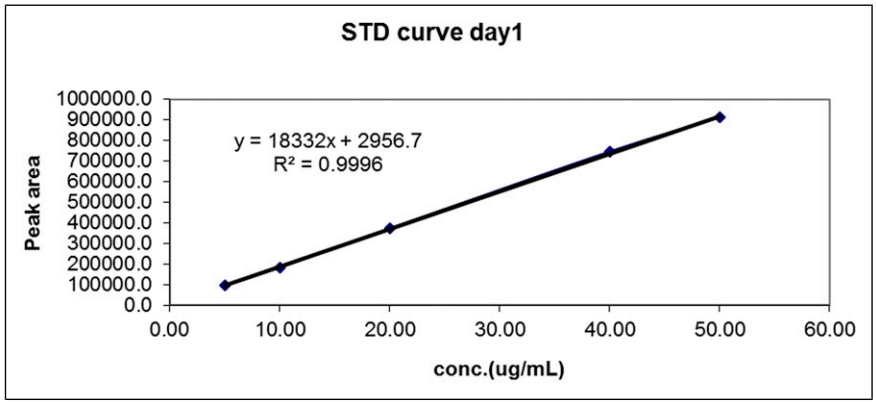

Figure 1. The calibration curve used to determine imipenem concentration.

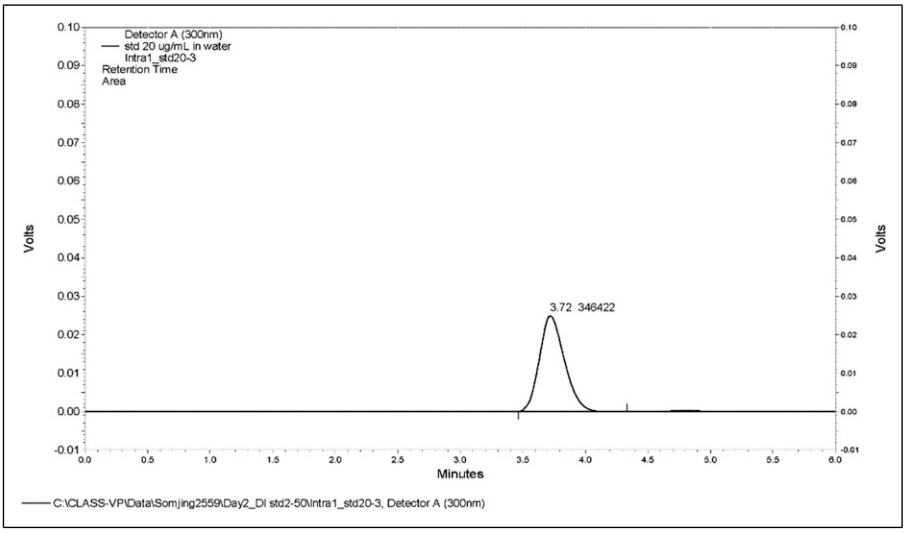

Figure 2. An example of chromatogram of standard imipenem at the concentration of 20 mcg/mL.

Pharmacokinetic/pharmacodynamic data[5] in critically ill patients with nosocomial pneumonia suggest that imipenem should be administered by standard infusion (1 g imipenem/1 g cilastatin over 30 minutes every 8 hours) or extended infusion with a decreased total dose (.5 g imipenem/0.5 g cilastatin over 3 hours every 6 hours). With this administration, imipenem concentrations were detected above the threshold concentration (≥40%) throughout the dosing interval in every patient group. Therefore, these results confirmed that extended infusion of .5 g (5 mg/mL) and 1 g (10 mg/mL) of imipenem could provide serum concentration above the MIC of MDR gram-negative pathogenic bacteria.[5] However, maintaining the stability of imipenem throughout 3-hour infusion is challenging, especially when it is given at room temperature in a tropical country (32 to 37°C).[5] Moreover, there is limited information about stability of imipenem for extended infusion at high temperature and the concentrations used in clinical practice. The objective of this study therefore was evaluation of imipenem stability for extended infusion in various concentrations and temperatures.

Material and Methods

Drugs, Chemicals, and Instruments

This study used commercially imipenem powder for IV injection available in .5-g vials from 2 different companies (brand A and brand B). The reference standard of imipenem trihydrate was a product of Fluka. The .9% sodium chloride solution in PVC bag was a product of GHP, Thailand. Potassium dihydrogen phosphate was purchased from BDH Laboratory Supplies. Orthophosphoric acid was from Merck. All solutions that were used in HPLC analysis were HPLC-grade, including acetonitrile and water from RCI lab scan.

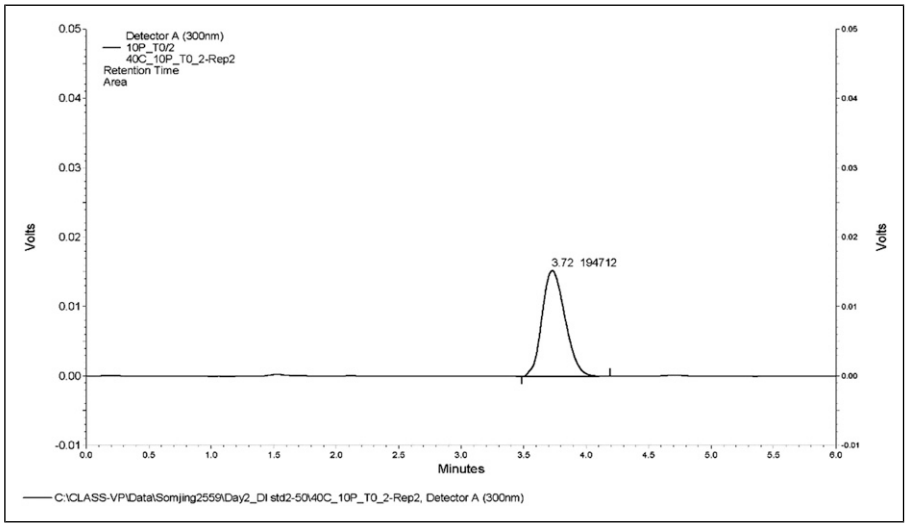

Figure 3. An example of chromatogram of 10 mcg/mL imipenem mL collected at 0 hour and 40°C.

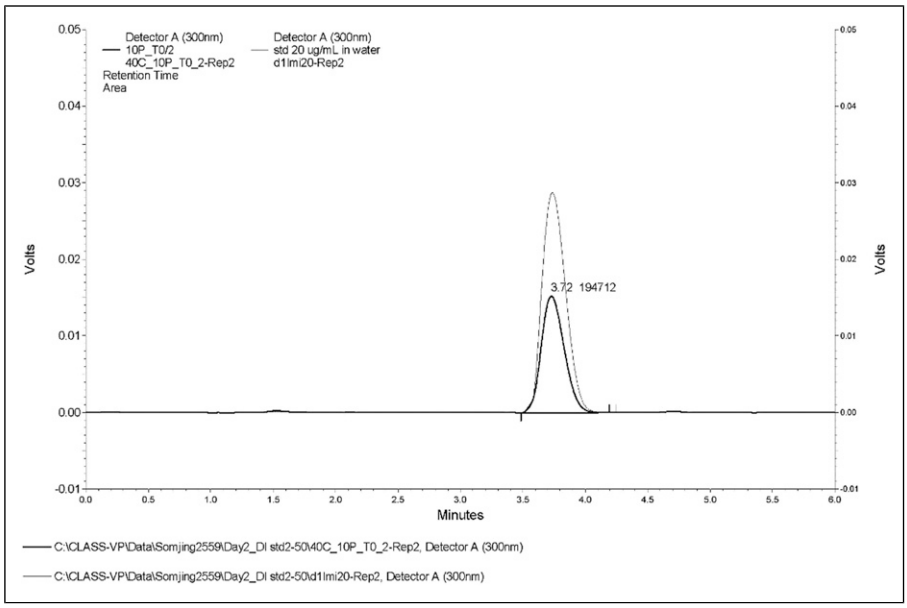

Figure 4. An example of chromatograms of imipenem at concentration of 10 mcg/mL collected at 0 hour and 40°C (solid black line) and standard imipenem at concentration of 20 mcg/mL (black dashed line).

Instruments and analytical methods were summarized as following: The assay for detection of imipenem concentrations was high performance liquid chromatography method. The HPLC was equipped with LC-20AD pump, a UV/Vis detector, au-to-sampler, column oven, degassing unit, and an LC solution integrator. This study used reverse-phase technique for HPLC analysis.[6-8] A 5 µm particle size C18 column with dimension of 250 × 4.0 mm was used. Imipenem injection was eluted isocratically at flow rate of 1.0 mL/min, using a mobile phase consisting of 95:5 v/v ratio of acetonitrile and 1 mM KH2PO4 buffer (pH 6.8). The UV/Vis detector was set at 300 nm. The HPLC system was operated at 25°C. Samples in this study were incubated at 25°C, 30°C and 40°C in a temperature and humidity controller cabinet, LHL-112 model.

Preparation of Imipenem Solutions and Analytical Method

Preparation of imipenem sample solution. Imipenem at the concentrations of 5 and 10 mg/mL in normal saline solution was tested for its stability after incubation for 0, 1, 2, 3, 4,

Table 1. The Mean Percentage ±SD of Imipenem at 25°C Compared with Initial Concentration.

	Imipenem Concentration (%) Mean ± S.D.			
	5 mg/mL		10 mg/mL	
Time (Hours)	Brand A	Brand B	Brand A	Brand B
0	100.00	100.00	100.0	100.0
1	97.61 ± 1.01	93.49 ± .32	95.84 ± 13.87	96.99 ± 4.38
2	103.92 ± .21	99.97 ± .03	83.02 ± .21	96.95 ± 6.05
3	95.02 ± 9.01	100.01 ± .08	90.57 ± 7.11	96.69 ± 3.96
4	101.60 ± .44	95.91 ± 3.28	84.71 ± 6.46	92.54 ± 3.28
6	100.82 ± .22	94.20 ± .94	84.71 ± 8.98	94.62 ± 1.20

Table 2. The Mean Percentage ±SD of Imipenem at 30°C Compared with the Initial Concentration.

	Imipenem Concentration (%) Mean ± S.D.			
	5 mg/mL		10 mg/mL	
Time (Hours)	Brand A	Brand B	Brand A	Brand B
0	100.00	100.00	100.0	100.0
1	95.35 ± 19.43	100.26 ± 5.62	81.93 ± 1.62	86.50 ± 3.86
2	92.39 ± 6.37	108.01 ± .87	76.31 ± 1.27	73.68 ± 1.05
3	91.63 ± 2.98	96.55 ± 9.89	76.23 ± 4.66	76.46 ± .43
4	94.72 ± .71	99.42 ± 2.72	69.81 ± 2.27	73.70 ± 5.40
6	96.93 ± 6.40	103.00 ± .52	77.67 ± 5.58	82.47 ± 2.94

Table 3. The Mean Percentage ±SD of Imipenem at 40°C Compared with the Initial Concentration.

	Imipenem Concentration (%) Mean ± S.D.			
	5 mg/mL		10 mg/mL	
Time (Hours)	Brand A	Brand B	Brand A	Brand B
0	100.00	100.00	100.0	100.0
1	101.25 ± 1.35	106.40 ± .39	88.24 ± 3.52	71.43 ± 4.66
2	102.40 ± 6.36	102.27 ± 1.07	93.96 ± 2.61	77.04 ± 8.86
3	100.15 ± 2.47	91.99 ± 10.59	77.11 ± 1.64	73.35 ± 5.10
4	93.37 ± .76	95.61 ± .80	86.77 ± 8.11	76.95 ± 8.48
6	92.64 ± 6.96	98.43 ± 3.91	62.60 ± 6.17	59.95 ± 18.95

and 6 hours at 25, 30, and 40°C.[6,9,10] Preparation of imipenem samples was as the following. Each .5-g vial was reconstituted with 20 mL of HPLC-grade water. A 4 mL of the reconstituted solution was added to 6 mL of .9% sodium chloride to make a 10 mg/mL sample, and a 2 mL of the reconstituted was added to 8 mL of .9% sodium chloride to make a 5 mg/mL sample. Three replicates of the 2 mL of 5 or 10 mg/mL in test tubes were prepared for each concentration and temperature, and 2 replicates of each prepared sample were injected for analysis.[6] The collected samples at each time point were diluted with HPLC-grade water to 10 mcg/mL. An aliquot of 20 µL was injected into the analytical column. Lastly, the concentration of the injected sample was calculated by the calibration curve.

Preparation of reference standard solution and calibration curve. A 5.0 mg Imipenem reference standard was weighed accurately, added to a 25 mL volumetric flask, and then dissolved with HPLC-grade water as a stock solution. A .25 mL aliquot of stock solution was diluted to 10 mL in HPLC-grade water in order to yield 5 µg/mL. The final

Table 4. Differences Between Percentages of 5 and 10 mg/mL Imipenem Solutions that were Stored for 6 hours Compared with the Initial Concentration.

	Imipenem Concentration (%) Mean ± S.D.					
	Brand A			Brand B		
Temperature (°C)	5 mg/mL	10 mg/mL	*P*-Value*	5 mg/mL	10 mg/mL	*P*-Value*
25	100.82 ± .22	84.71 ± 8.98	.036	94.20 ± .94	94.62± 1.20	.658
30	96.93 ± 6.40	77.67 ± 5.58	.017	103.00 ± .52	82.47 ± 2.94	.001
40	92.64 ± 6.96	62.60 ± 6.17	.005	98.43 ± 3.91	59.95 ± 18.95	.026

* *P*-value by t-test to compare concentration of 5 and 10 mg/mL.

Table 5. Differences Between Percentages of Imipenem Solutions that were Stored at 25, 30, and 40°C for 6 hours Compared with the Initial Concentration.

Imipenem Concentration (%) Mean ± S.D.		Temperature (°C)			
		25	30	40	*P*-Value*
Brand A	5 mg/mL	100.82 ± .22	96.93 ± 6.40	92.64 ± 6.96	.284
	10 mg/mL	84.71 ± 8.98	77.67 ± 5.58[a]	62.60 ± 6.17[b]	.001
Brand B	5 mg/mL	94.20 ± .94	103.00 ± .52[a]	98.43 ± 3.91	.001
	10 mg/mL	94.62± 1.20	82.47 ± 2.94	59.95 ± 18.95[b]	.004

**P*-value by ANOVA to compare temperature of 25, 30, and 40°C.
P-value < .05 on the post-hoc analysis for temperature.
[a]25 vs 30°C.
[b]25 vs 40°C.

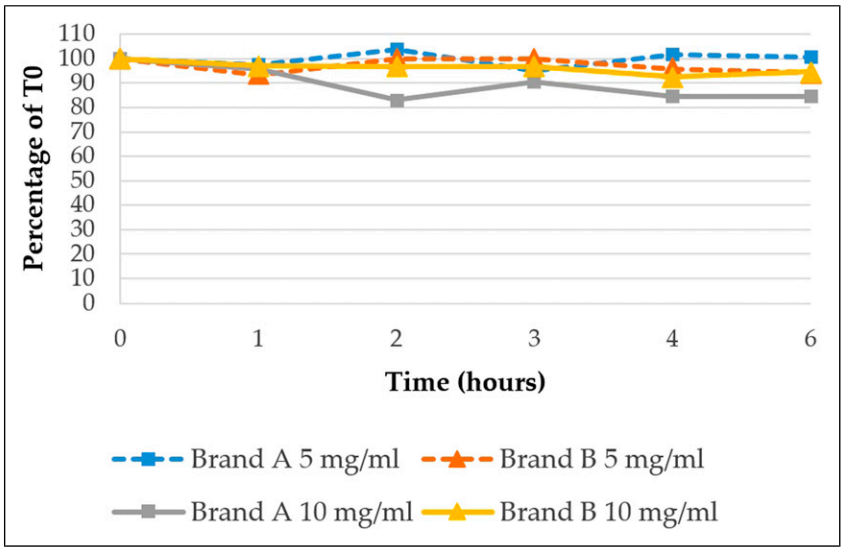

Figure 5. Stability of imipenem at 2 different concentrations in .9% sodium chloride solution incubated at 25°C for 6 hours.

concentrations of 10, 20, 40, and 50 µg/mL were prepared as previously described and were used to generate a standard curve. The calibration curve was plotted between peak area against imipenem reference standard in the concentration of 5 to 50 µg/mL.

The HPLC analytical method for imipenem used in this study was the same as the method used by Swanson et al,[11] Srinivasan et al,[12] and Tissel et al.[6] Therefore, was verified by determine the linearity of calibration curve, the precision and the accuracy when determine the imipenem concentration

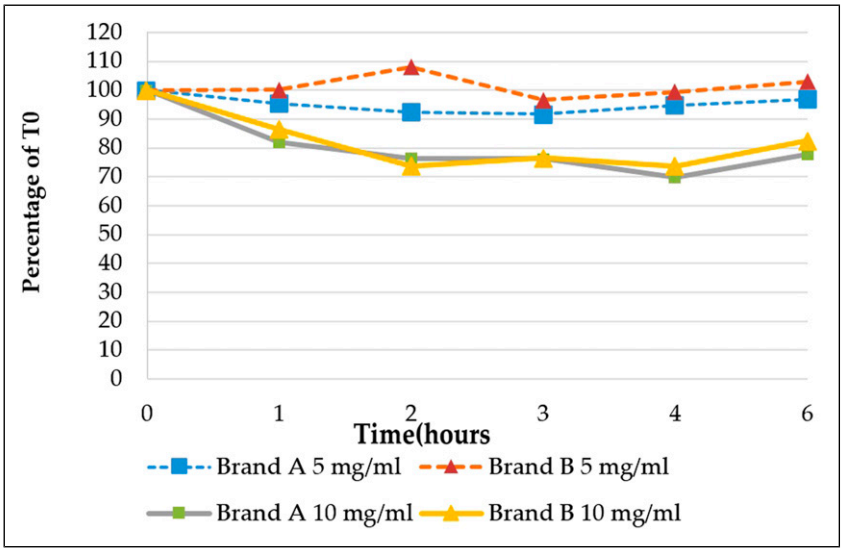

Figure 6. Stability of the 2 concentrations of imipenem (5 and 10 mg/mL) in .9% sodium chloride solution incubated at 30°C for 6 hours.

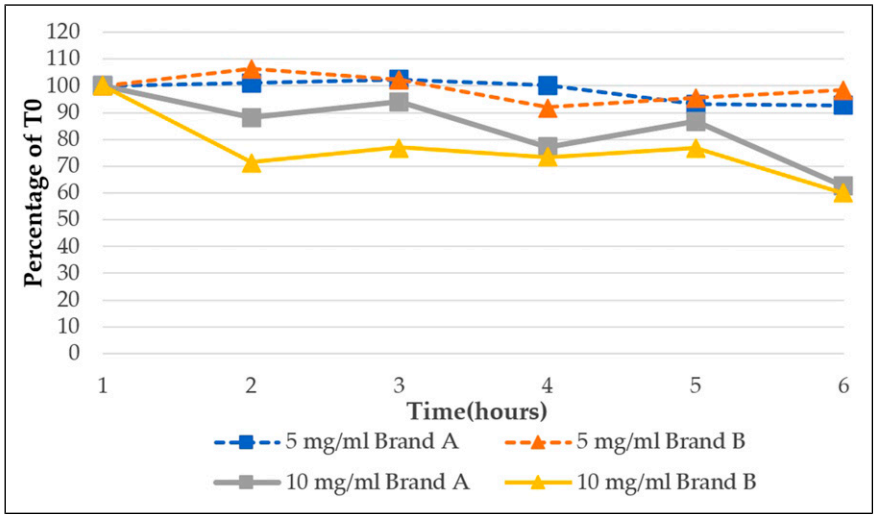

Figure 7. Stability of the 2 concentrations of imipenem in .9% sodium chloride solution incubated at 40°C for 6 hours.

between 5–50 µg/mL reference standard. The acceptance criteria of linearity, precision, and accuracy were $r^2 > .998$, % CV <10% and mean recovery 98-102%, respectively.[9,10]

Data Analysis

Results were reported as mean percentage with standard deviation of imipenem compared to the initial time point. Samples were considered stable if mean percentage of imipenem remains higher than 90% of the control (0 hour) according to the U.S. Pharmacopeia.[13] Difference between imipenem remaining at 6 hours and control solution was analyzed using a t-test with equal variance. Moreover, one-way ANOVA was used for the analysis of percentages of imipenem remaining at 6 hours on temperature of 25, 30, and 40°C, with a Bonferroni post-hoc analysis. The significance levels of all analysis were set at <.05. Data analysis was performed using Stata software, version 14 (Stata Corp, College Station, TX, USA).

Results

The calibration curve shows a linearity in the range of 5–50 µg/mL, with $r^2 > .99$.

Figure 1 presents the standard curve between peak area and concentration. Both within run precision (%CV) and between run precision were less than 10%. The within run accuracy was 96.13–100.18% and the between run accuracy was 99.05–101.17%. The examples of chromatograms of standard imipenem at the concentration of 20 mcg/mL, of 10 mcg/mL imipenem collected at 0 hour and 40°C, and of both solutions of are shown in Figures 2–4, respectively.

(1) The stability of imipenem at 25°C

Brand A and B imipenem solutions at the concentration of 5 mg/mL were stable (>90%) for 6 hours when stored at 25°C (Table 1). For brand A, the 10 mg/mL solution was stable for up to 3 hours at 25°C. However, the 10 mg/mL solution of brand B was stable for up to 6 hours at 25°C (Table 1).

(2) The stability of imipenem at 30°C

Brand A and B imipenem solutions at the concentration of 5 mg/mL were stable (>90%) when stored at 30°C for up to 6 hours (Table 2). However, the 10 mg/mL solutions of both brands were stable less than 1 hours at 30°C (Table 2).

(3) The stability of imipenem at 40°C

Brands A and B imipenem solutions at the concentration of 5 mg/mL were stable for 6 hours (>90%) when stored at 40°C (Table 3). However, neither brand A nor B at the concentration of 10 mg/mL was stable at 1 hour when incubated at 40°C (Table 3).

The percentages of imipenem remaining at 6 hours compared to the baseline are described in Tables 4 and 5 with significance between the concentrations of 5 and 10 mg/mL and between the temperatures of 25, 30, and 40°C. Brand A and B imipenem solutions at the concentration of 10 mg/mL were significantly decreased when stored at 40°C for 6 hours compared with 25°C. Moreover, the significant decreases of 10 mg/mL of imipenem solutions stored at 25, 30, and 40°C for 6 hours were observed, as compared with 5 mg/mL of imipenem.

The degradation of the brand A and B imipenem solutions (Figures 5–7) was therefore considered concentration and temperature dependent.

Discussion

Based on the definition of stability, admixtures are stable if mean percentages of imipenem remain greater than 90% of baseline. According to the results, the 5 mg/mL imipenem solutions of brand A and B were found stable for 6 hours at 25°C, 30°C, and 40°C, respectively. At 25°C, the 10 mg/mL solutions were stable for up to 3 hours for brand A and 6 hours for brand B. However, brands A and B imipenem solutions at the concentration of 10 mg/mL were stable for less than 1 hour at 30°C and 40°C. Therefore, the degradation of imipenem solution was depending on both concentration and temperature.

Extended infusion is the optimal administration to achieve the best PK/PD properties of imipenem, so imipenem should be administered by extended infusion in clinical practice.[5] Nevertheless, this study found that the stability of reconstituted imipenem was influenced by temperature. Keel, et al[14] confirmed that 5 mg/mL imipenem had 10% degradation in 6, 4 and 3 hours at 30, 35, and 40°C, respectively. In agreement with Viaene, et al,[15] the stability of imipenem was influenced by temperature (10% degradation at 25°C and 37°C after 3.5 and 2.75 hours, respectively). However, the concentrations used in the mentioned studies were different from this study since this study focused on the 2 concentrations that were generally used in real clinical practice. Also, there were no data suggesting the role of concentration in imipenem stability.[11] To the best of our knowledge, the association between degradation rate of imipenem and its concentration was firstly reported in this study. This study suggested that stability of imipenem was much decreased at high concentration and high temperature. The percentages of imipenem in the preparation of 10 mg/mL showed more than 10% reduction after 1 hour of incubation at 30 and 40°C.

According to these data, the factors influencing stability of imipenem in normal saline solution were drug concentration and temperature. When imipenem is administered by extended infusion, it is recommended to be diluted to low concentration (≤5 mg/mL); higher concentration such as 10 mg/mL should be administered by infusion for no longer than 3 hours or infused in air-conditioned room (≤25°C) if possible, especially in tropical countries.

This study still had some limitations. First, this experiment was performed at the indicated temperatures (25, 30, and 40°C), so the results should not be extrapolated to out-of-range temperature such as <25°C or >40°C. Moreover, because normal room temperature in tropical countries is 32–40°C, stability tests for higher temperature should be performed in further studies. Lastly, these results were from the specific brands of imipenem for injection and other brands may have different imipenem salts or ingredients, so the application of stability results from this study to other brands of imipenem should be done with care.

Conclusions

The results from this study indicated that imipenem stability correlated with temperature and concentration. In particular, the higher temperature and drug concentration contributed to the lower stability of imipenem. If imipenem has to be administered by extended infusion, the solution should be diluted to the lowest concentration as possible and infused at low temperature such as in air-conditioned room.

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Author Contributions

Conceptualization, W.K.; Data curation, W.K., C.S. and P.V.; Formal analysis, W.K. and C.S.; Investigation, W.K., C.S. and P.V.; Methodology, W.K., C.S. and P.V.; Project administration, W.K.; Software, W.K. and C.S.; Validation, P.V., S.O.; Writing—original draft, W.K., C.S. and P.V.; Writing—review & editing, W.K. and S.U.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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