Evaluation of the stability of temocillin in elastomeric infusion devices used for Outpatient Parenteral Antimicrobial Therapy in accordance with the requirement of the UK NHS Yellow Cover Document

Study Report

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Summary

Objectives: The objective of the study was to evaluate the stability of temocillin solution in two elastomeric infusion devices (B. Braun Easypump® II LT 270-27- S and Spirit Medical Dosi-Fusor® L25915-250D1) for OPAT administration during 14 days of 5°C ± 3°C fridge storage followed by 24 hours exposure at in-use temperature of 32°C, when reconstituted with 0.3% citrate buffer at pH7.

Methods: Stability testing was conducted in accordance with the standard protocols for deriving and assessment of stability of small molecule aseptic preparation recommended by the National Health Service (NHS) Pharmaceutical Quality Assurance Committee- also known as the Yellow Cover Document (YCD). Stability indicating assay method was developed with an ultra-high-performance liquid chromatography (UHPLC) system using photodiode array detector. Temocillin concentrations corresponding to low (500 mg/240mL), intermediate (4000 mg/mL) and high (6000 mg/240 mL) concentration were tested in triplicate devices with duplicate samples taken at 11 time points during 14 days of fridge storage followed by 24 hours at the in-use temperature exposure of 32°C.

Results: A total of 396 samples were collected and assayed. The percentage of temocillin remaining after 14 days of fridge storage was greater than 97% in both devices and at all concentrations tested. During in-use temperature, 95% stability limit was achieved for 12 hours for all doses and devices tested except for the high concentration in the Dosi-Fusor device which met this criterion for only10 hours of in-use temperature exposure. However, for all devices and dose tested, the degradation of temocillin was <9% at the end of the 24 hours in-use temperature exposure.

Conclusion: Temocillin reconstituted with 0.3% citrate buffer at pH 7 in elastomeric infusion devices can be stored in a fridge (2-8°C) for two weeks meeting the YCD acceptance criteria of <5 % degradation. The current data supports twice daily dosing of temocillin with <5% degradation at in-use temperature of 32°C for 12 hours. However, in jurisdictions where <10% degradation limit is acceptable, once daily dosing with 24-hour continuous infusion is feasible with <9% degradation. Future studies should evaluate the clinical exposures achieved from once daily and twice daily temocillin regimens when administered using elastomeric infusion devices.

1. Background

Temocillin is a semi-synthetic derivative of penicillin initially designed to achieve high stability to beta-lactamase enzymatic degradation while maintaining activity against a broad spectrum of Gram-negative bacteria [1,2]. In early *in vitro* studies, it demonstrated good activity against a range of Gram-negative bacteria including those producing commonly encountered plasmid and chromosomal beta-lactamases [2,3]. However, it lacked activity against most *Pseudomonas aeruginosa* strains, other *Pseudomonas spp*, *Acinetobacter spp*., anaerobes and Gram-positive organisms [4]. Its spectrum appeared restricted mainly to Enterobacterales and due to the perceived narrow spectrum, it was abandoned after its initial development in the 1980s [5], largely remaining as an orphan drugs for *Burkholderia species* [6].

Interest in reviving the old temocillin increased since 2009 with the growing effort to repurpose old, forgotten antibiotics for resistant infection. Temocillin has a potential role as an alternative treatment for resistant infections particularly due to its excellent stability against extended spectrum beta-lactamases (ESBL) and AmpC enzymes [7-9]. In ongoing studies, due to its stability against ESBLs and AmpC enzymes [8,9] that are primarily responsible for third generation-cephalosporin resistant Enterobacterales (3GCR-E), temocillin is being considered as a potential alternative to carbapenems for 3GCR-E infections [10]. Although previous retrospective studies have demonstrated its potential effectiveness against multi-drug resistant Enterobacterials, data from randomised controlled trials is still pending [10-12].

Currently, temocillin is mainly used in Europe, where it is recommended for the treatment of septicaemia, urinary tract infections, and lower respiratory tract infections caused by susceptible Gram -negative bacilli [5]. In this context, it is potentially useful as a carbapenem sparing agent when resistance to other commonly indicated antibiotics is observed [7,13]. Its narrow spectrum of activity can also be leveraged for directed therapy, without the risk of triggering resistance selection for Gram-positives, anaerobes, and most non-fermenting Gram-negatives including *Acinetobacter baumanii* and *P. aeruginosa* [13]. It is also advantageous to minimise the risk of colonisation with *Clostridioides difficile* or third generation cephalosporin resistant Enterobacterales; a recent randomised multicentre trial comparing temocillin and cefotaxime in adults with febrile urinary tract infections demonstrated that temocillin exhibited less selective pressure [14].

Outpatient Parenteral Antimicrobial Therapy (OPAT) is being increasingly used for conditions including urinary tract and lower respiratory infections when oral alternatives appropriate for the suspected/confirmed pathogen are unavailable [15-17]. Where drug stability allows, once daily dosing of antibiotics for OPAT using elastomeric devices can be a convenient way of delivering treatment. Temocillin has been identified as one of the drugs suitable for OPAT use both due to the increased exposure achieved from continuous infusion of its total daily dose,

and due to its clinical indications. Several studies have shown continuous infusion administration of temocillin to achieve favourable exposures that cover for the high clinical breakpoint targets of up to 16 mg/L[18-20]. Currently, temocillin is being used in European OPAT programs; in some jurisdictions, for example, it is the most frequently used OPAT antibiotics for the treatment of urinary tract infection [21].

Although it is being used for OPAT , as for most other antibiotics, stability data for temocillin in elastomeric devices, that is compliant with the UK national standards for stability testing as outlined in the Yellow Cover Document (YCD), is not yet available [22]. Carryn *et al*. [23] assessed temocillin stability in two elastomeric infusion pumps during long term fridge storage (4 weeks) and 24 hour room temperature storage at a concentration of 10 or 20 g/L in water for injection. They showed that temocillin stability was greater than 90% during both fridge storage and 24 hours exposure at room temperature. However, this study does not comply with the testing protocols of the YCD; for example, concentrations did not cover for all clinically relevant concentrations, the maximum in-use temperature conditions (32 °C) was not tested, and analysis of sub-visible particles was not conducted.
This study was therefore conducted to evaluate the stability of temocillin in two commonly used elastomeric infusion devices (Easypump® II LT 270-27-S and Dosi-Fusor® L25915-250D1), in accordance with the YCD testing protocol recommendations to provide data for OPAT services.

2. Analytical Method

The stability indicating assay method was adapted from the method of Kahsay et al, 2014 [24].

Materials and Methods
Solvents used with HPLC or LCMS grade: Acetonitrile HPLC Lichrosolv (Merck, Darmstadt, Germany) and Methanol Optima LCMS Grade (Fisher Chemicals, Fair Lawn, USA). Disodium Hydrogen Phosphate was AR grade from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q Direct water purification system. Pure temocillin disodium was obtained from Alsachim (Illkirch, France).

Chromatographic apparatus and conditions
The stability indicating assay method was conducted with a Nexera2 ultra-high-performance liquid chromatography (UHPLC) system comprising two LC30AD pumps with degassers, SIL-30AC autosampler, CTO-30AD oven and SPD-M30A photodiode array detector, controlled by LabSolutions software (Shimadzu, Kyoto, Japan).

The stationary phase was a Symmetry C18, 100 x 2.1 mm (3.5 µm) analytical column (Waters, Milford, USA) preceded by a SecurityGuard Ultra C18 guard column (Phenomenex, Torrance, USA) held at 30°C. Mobile phase A was 30 mM sodium phosphate buffer solution at pH 7. Mobile phase B was Acetonitrile/Methanol/Water (50/10/40, v/v/v). Delivery of mobile phase was by gradient at 0.2 mL/min.

The autosampler was held at 4°C, and injection volume was 0.5 µL. The PDA detector scanned from 200 to 400 nm, and quantification was at 235 nm.

Solutions for Analysis

Temocillin reference material was prepared in mobile phase A (30 mM sodium phosphate buffer solution at pH7) to concentrations of 560, 640, 720, 800, 880, 960 µg/mL as calibration standards. Quality controls were prepared by dissolving test formulation in test buffer to test concentrations of 25.0, 16.6 and 2.08 mg/mL). Aliquots were stored at -80°C until use.
In an assay batch test samples and quality controls were diluted in mobile phase A to a bring the nominal concentration to 800 µg/mL. Diluted test samples and QCs were injected with a set of calibrators. Samples for injection were held at 4°C and all batches with injected over less than 24 hours.

Validation of the HPLC method

The area of the two epimer peaks for temocillin were summed to give a temocillin area. A straight-line calibration curve was generated from temocillin peak area (no weighting, not forced through zero). Detector linearity was demonstrated from 560 to 960 µg/mL with r2 values of 0.9990, 0.9970 and 0.9987. Slopes were 2309, 2268 and 2536, respectively. The precision and accuracy of the assay was assessed from duplicate analysis quality controls at each of the test concentrations across 3 batches. Precision was 0.9%, 1.3% accuracy was -0.4%, 0.9% and -0.6% at and 1.6% at 2.08, 16.6 and 25.0 mg/mL, respectively.

Forced degradation was undertaken using 0.1 M hydrochloric acid, 0.1 M sodium hydroxide and 3% hydrogen peroxide. Temocillin was tested at 10 mg/mL at room temperature and at 50°C over 3 hours. Degradation peaks from acidic degradation (n=3), basic degradation (n=4) and oxidation (n=6) eluted separate from temocillin. For the analysis of test samples only 5 degradation peaks were present: designated A, B, C D and E (Figure 1).

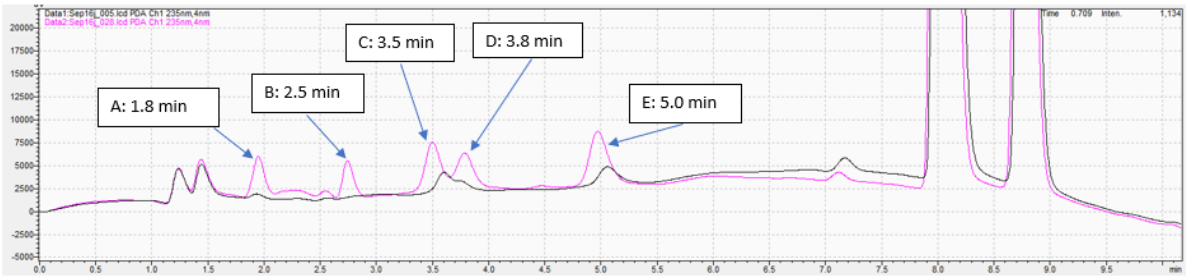

Figure 1. Temocillin chromatogram showing five major degradation peaks A, B, C, D and E.

3. Diluent

The diluent used was 0.3% citrate buffer at pH7. This diluent was selected based on a pre-scoping study of temocillin stability in various solvents. A commercial buffer recipe provided by BSAC was adapted to prepare the required volume of 0.3% w/v Citrate buffer pH7 in-house from trisodium citrate dihydrate (Sigma-Aldrich, Lot # SLCG13355) and citric acid anhydrous (Sigma-Aldrich, Lot # STBJ8530).

4. Container

Two elastomeric infusion devices from different manufacturers were used. Easypump® (B. Braun, Sheffield, UK) and Dosi-Fusor® (Spirit Medical, Derby, UK.

i) Easypump® II LT 270-27- S, volume=270 mL, Flow rate= 10mL/hr (B. Braun, Sheffield, UK, Lot # 19E29GE221)

ii) Dosi-Fusor® L25915-250D1, volume=250 mL, Flow Rate=10.4 mL/hr (Spirit Medical Limited, Derbyshire, UK, Lot # 211005L)

5. Concentrations

Three concentrations of temocillin were tested. These concentrations were selected to cover the clinical range of doses (concentrations); they correspond to low, intermediate, and high daily doses used clinically when the devices are filled to the volume of 240 mL:

i) Low dose (500 mg/240 mL = 2.17 mg/mL)

ii) Intermediate dose (4000 mg/240 mL= 16.67 mg/mL)

iii) High dose (6000 mg/ 240 mL=25 mg/mL)

6. Storage Protocol

Table 1 below summarises the storage and in-use temperature conditions used for the study. Devices filled with temocillin were stored in refrigerator (2 to 8°C) for 14 days without exposure to UV light. Each device was wrapped with aluminium foil to completely cover its surfaces and prevent exposure to light during storage and sampling. Following the 14 days of refrigeration, the devices were stored within an incubator at the maximum expected in-use temperature of 32°C for 24 hours [26].

Table 1. Temperature conditions

	Condition	Temperature	Duration
1	Refrigerated without exposure to UV light	5°C ± 3°C	14 days
2	In use solution temperature without exposure to UV light	32°C	24 hours

7. Sample numbers

Duplicate samples were collected from each individual device for the two device types that were tested at three concentrations in triplicate devices, at 11 different time points which include at 0, 24, 48, 96, 168, 240 and 336 hours during refrigeration and at 340, 344, 356, and 360 hours at the subsequent in-use 32°C running phase.
The whole test was run in one batch. A total of 396 samples were collected.

8. Testing Protocol

The testing protocol was developed in accordance with the YCD recommendations.

9. Results

9.1 Colour, clarity, and precipitation

Samples were collected from each device for visual analysis of colour, clarity, and any precipitation at similar time points of sampling for concentration measurement. No visible precipitation was observed for all sample from both device during fridge storage and in-use temperature. Similarly, all samples appeared clear with no visible turbidity. All samples appeared colourless during fridge storage. However, during in-use temperature, sample taken from devices filled with intermediate and high dose had a faint yellowish appearance. All devices filled with the low dose appeared colourless during both in-use and fridge storage.

9.2 pH

Table 2. Change in pH of temocillin solution in Easypump® II LT 270-27- S elastomeric devices filled at low, intermediate, and high dose during fridge and in-use temperature storage

Temperature Condition	Time	Mean ± SD observed pH and change in mean pH from baseline by dose					
		Low Dose		Intermediate Dose		High Dose	
		Mean ± SD	? pH	Mean ± SD	? pH	Mean ± SD	? pH
Fridge storage (5°C +/- 3°C)	0	6.98 ± 0	0	6.84 ± 0	0	6.81 ± 0	0
	24	7 ± 0.03	0.0	6.8 ± 0.02	0.0	6.78 ± 0.01	0.0
	48	7.02 ± 0.04	0.0	6.89 ± 0.01	0.1	6.84 ± 0.03	0.0
	96	7 ± 0	0.0	6.84 ± 0	0.0	6.83 ± 0.02	0.0
	168	7 ± 0.01	0.0	6.83 ± 0.01	0.0	6.81 ± 0.01	0.0
	240	6.97 ± 0.01	0.0	6.83 ± 0.02	0.0	6.78 ± 0	0.0
	336	6.96 ± 0	0.0	6.8 ± 0	0.0	6.73 ± 0.01	-0.1
In-use temperature (32°C)	340	6.8 ± 0.01	-0.2	6.65 ± 0.01	-0.2	6.61 ± 0	-0.2
	344	6.64 ± 0.02	-0.3	6.57 ± 0.03	-0.3	6.46 ± 0	-0.4
	356	6.51 ± 0.02	-0.5	6.37 ± 0.03	-0.5	6.3 ± 0.02	-0.5
	360	6.46 ± 0.01	-0.5	6.32 ± 0.02	-0.5	6.26 ± 0.02	-0.5

Table 3. Change in pH of temocillin solution in Dosi-Fusor® L25915-250D1S elastomeric devices filled at low, intermediate, and high dose during fridge and in-use temperature storage

Temperature Condition	Time	Mean ± SD observed pH and change in mean pH from baseline by dose					
		Low Dose		Intermediate Dose		High Dose	
		Mean ± SD	? pH	Mean ± SD	? pH	Mean ± SD	? pH
Fridge storage (5°C +/- 3°C)	0	6.98 ± 0	0	6.84 ± 0	0	6.81 ± 0	0
	24	6.97 ± 0.01	0.0	6.84 ± 0.02	0.0	6.82 ± 0.01	0.0
	48	6.98 ± 0	0.0	6.83 ± 0.02	0.0	6.8 ± 0	0.0
	96	6.98 ± 0.01	0.0	6.81 ± 0	0.0	6.77 ± 0.02	0.0
	168	6.96 ± 0	0.0	6.79 ± 0.01	0.0	6.75 ± 0	-0.1
	240	6.96 ± 0	0.0	6.79 ± 0.01	-0.1	6.73 ± 0.01	-0.1
	336	6.97 ± 0.01	0.0	6.77 ± 0.01	-0.1	6.71 ± 0	-0.1
In-use temperature (32°C)	340	6.88 ± 0	-0.1	6.69 ± 0	-0.2	6.63 ± 0.01	-0.2
	344	6.84 ± 0.01	-0.1	6.64 ± 0.01	-0.2	6.58 ± 0	-0.2
	356	6.67 ± 0.02	-0.3	6.48 ± 0.01	-0.4	6.39 ± 0	-0.4
	360	6.62 ± 0.01	-0.4	6.43 ± 0	-0.4	6.34 ± 0.01	-0.5

9.3 Temocillin concentration

9.3.1 Low dose (500 mg in 240 mL; 2.17mg/mL)

Table 4 summarises the mean percentage of temocillin remaining at each of the sampling time points for Easypump® II LT 270-27-S and Dosi-Fusor® L25915-250D1 devices filled to 240 mL final volume with the low daily dose of 500 mg temocillin; initial concentration of 2.17 mg/mL.

Table 4. Mean percent remaining for the low dose (500 mg/240 mL = 2.17 mg/mL) by device

Temperature Condition	Time (h)	Mean ± SD of percent remaining by device type	
		Easypump® II LT 270-27- S	Dosi-Fusor® L25915-250D1
		Mean ± SD (%)	Mean ± SD (%)
Fridge storage (5°C +/- 3°C)	0	100 ± 0	100 ± 0
	24	99.7 ± 0.5	100.1 ± 1.1
	48	99.8 ± 0.6	99.2 ± 1.6
	96	99.6 ± 0.3	98.8 ± 1.1
	168	99.3 ± 0.4	98.4 ± 1
	240	98.6 ± 0.2	98.4 ± 1.5
	336	97.9 ± 0.5	98.7 ± 1.2
In-use temperature (32°C)	340	97.7 ± 0.7	98.4 ± 1.3
	344	97.1 ± 0.6	97.4 ± 1.2
	356	94.2 ± 1	94.4 ± 1.6
	360	92.9 ± 0.3	93.5 ± 0.9

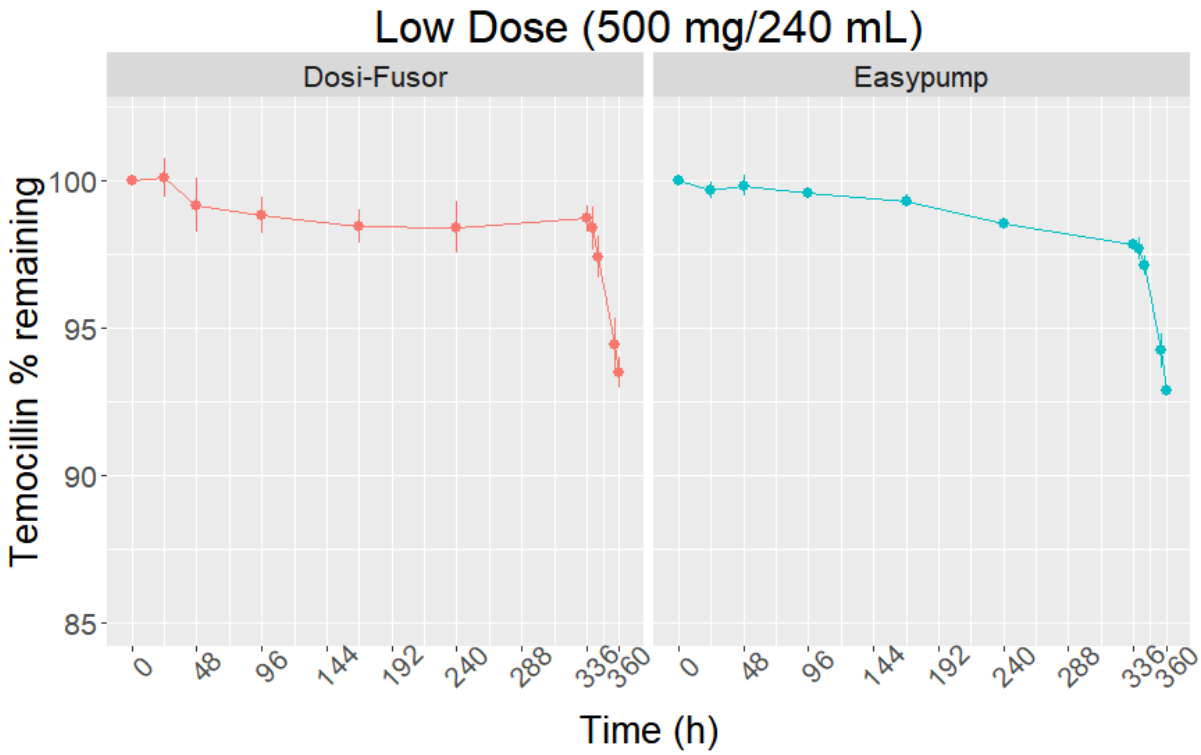

Figure 2. Percentage of temocillin remain during fridge storage (5°C +/- 3°C) from time 0 to 336 hour followed by in-use temperature storage at 32 °C from 336 to 360 hours (at a low dose of 500 mg in 240 mL; 2.17 mg/mL).

9.3.2 Intermediate dose (4000 mg in 240 mL; 16.67 mg/mL)

Table 5. Mean percent remaining for the intermediate dose (4000mg/240 mL = 16.67 mg/mL) by device

Temperature Condition	Time (h)	Mean ± SD of percent remaining by device type	
		Easypump® II LT 270-27- S	Dosi-Fusor® L25915-250D1
		Mean ± SD (%)	Mean ± SD (%)
Fridge storage (5°C +/- 3°C)	0	100 ± 0	100 ± 0
	24	100.4 ± 0.8	99.7 ± 0.1
	48	99.7 ± 0.4	99.7 ± 0.3
	96	99.6 ± 0.8	99.3 ± 0.2
	168	99.2 ± 0.6	99 ± 0.1
	240	98.9 ± 0.7	98.3 ± 1.4
	336	97.5 ± 1.5	97.7 ± 0.6
In-use temperature (32°C)	340	97.5 ± 0.9	96.9 ± 0.9
	344	96.4 ± 0.7	95.9 ± 0.4
	356	93.7 ± 0.7	93.7 ± 0.3
	360	92 ± 1.4	92.4 ± 0.9

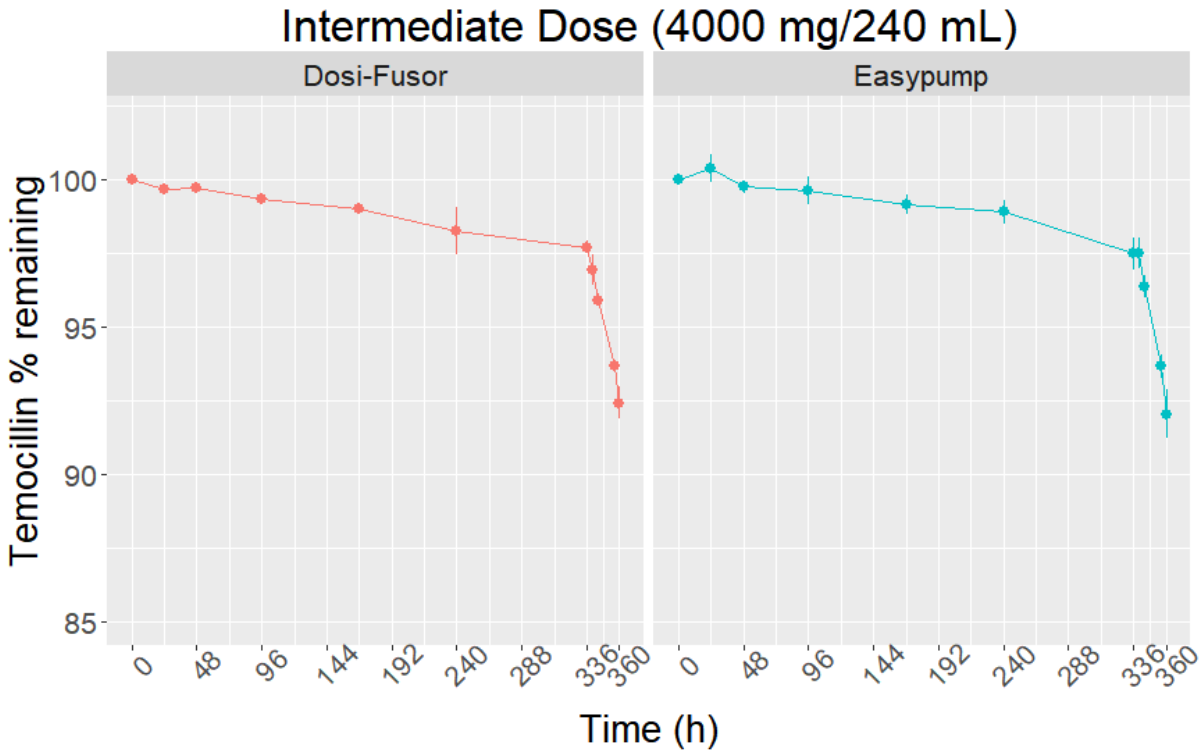

Figure 3. Percentage of temocillin remain during fridge storage (5°C +/- 3°C) from time 0 to 336 hour followed by in-use temperature storage at 32 °C from 336 to 360 hours (at an intermediate dose of 4000 mg in 240 mL; 16.67 mg/mL)

9.3.3 High dose (6000 mg in 240 mL; 25 mg/mL)

Table 6. Mean percent remaining for the high dose (6000mg/240 mL = 25 mg/mL) by device

Temperature Condition	Time (h)	Mean ± SD of percent remaining by device type	
		Easypump® II LT 270-27- S	Dosi-Fusor® L25915-250D1
		Mean ± SD (%)	Mean ± SD (%)
Fridge storage (5°C +/- 3°C)	0	100 ± 0	100 ± 0
	24	99.7 ± 0.7	99.7 ± 1.6
	48	99.7 ± 0.4	99.1 ± 0.2
	96	99.1 ± 0.6	99.1 ± 0.3
	168	98.8 ± 0.5	97.9 ± 1.4
	240	98.9 ± 0.3	97.6 ± 0.7
	336	97.5 ± 0.3	96.8 ± 1.3
In-use temperature (32°C)	340	97.4 ± 0.5	96.7 ± 1.1
	344	97 ± 1.8	95.7 ± 1.4
	356	93.3 ± 1.6	93.2 ± 0.9
	360	91.9 ± 1.8	91.1 ± 1.4

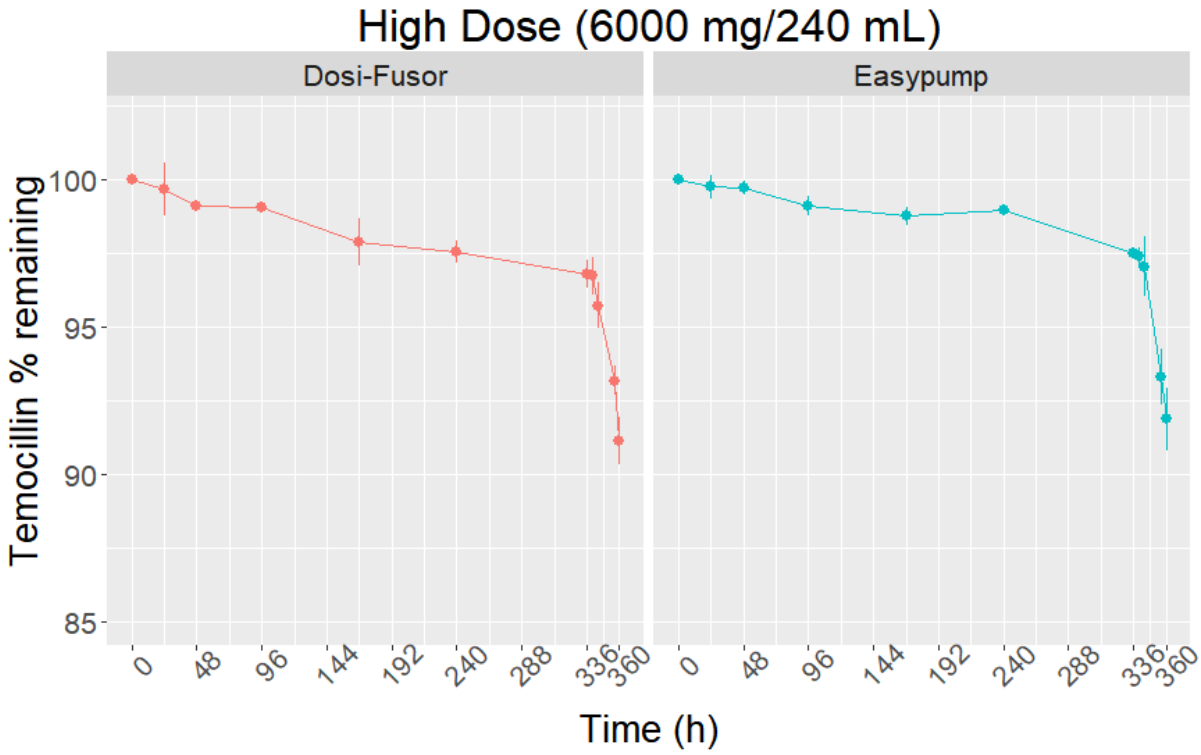

Figure 4. Percentage of temocillin remain during fridge storage (5°C +/-3°C) from time 0 to 336 hour followed by in-use temperature storage at 32°C from 336 to 360 hours (at high dose of 6000 mg in 240 mL; 25 mg/mL)

9.3.4 Comparison of stability by dose, device type and storage condition

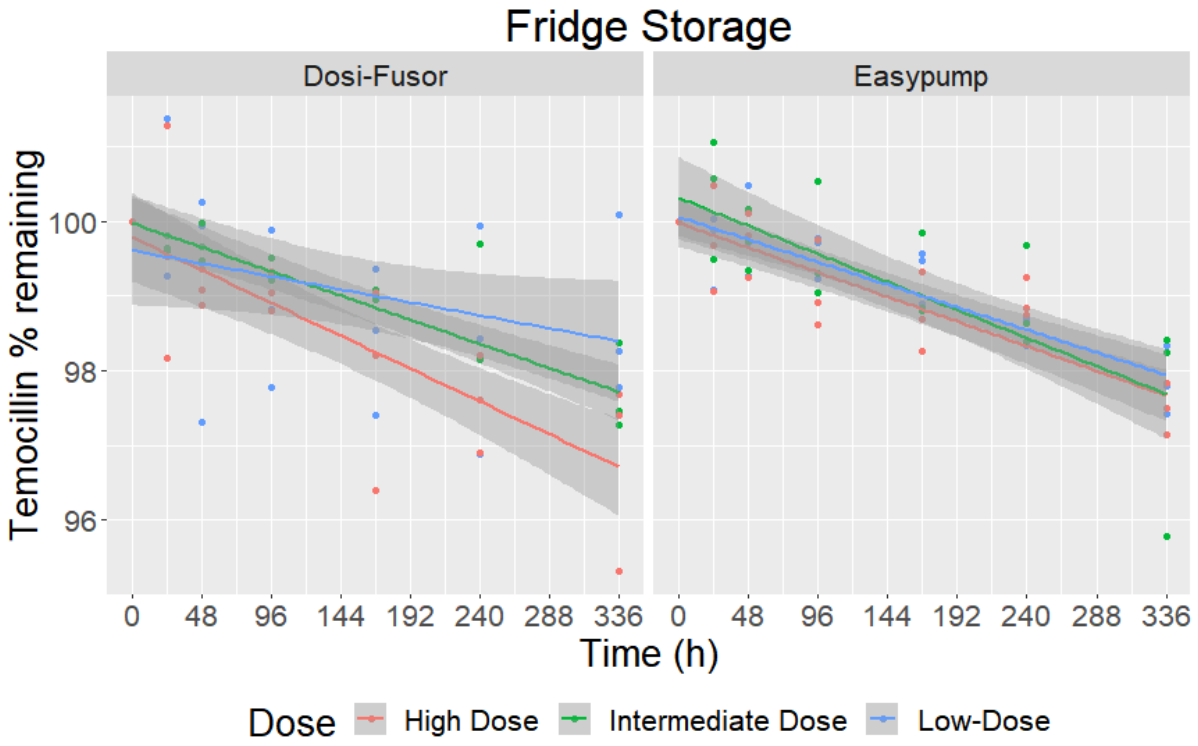

Figure 5. Comparison of percentage of temocillin remaining during fridge storage (5°C +/-3°C) for 14 days by dose and device type

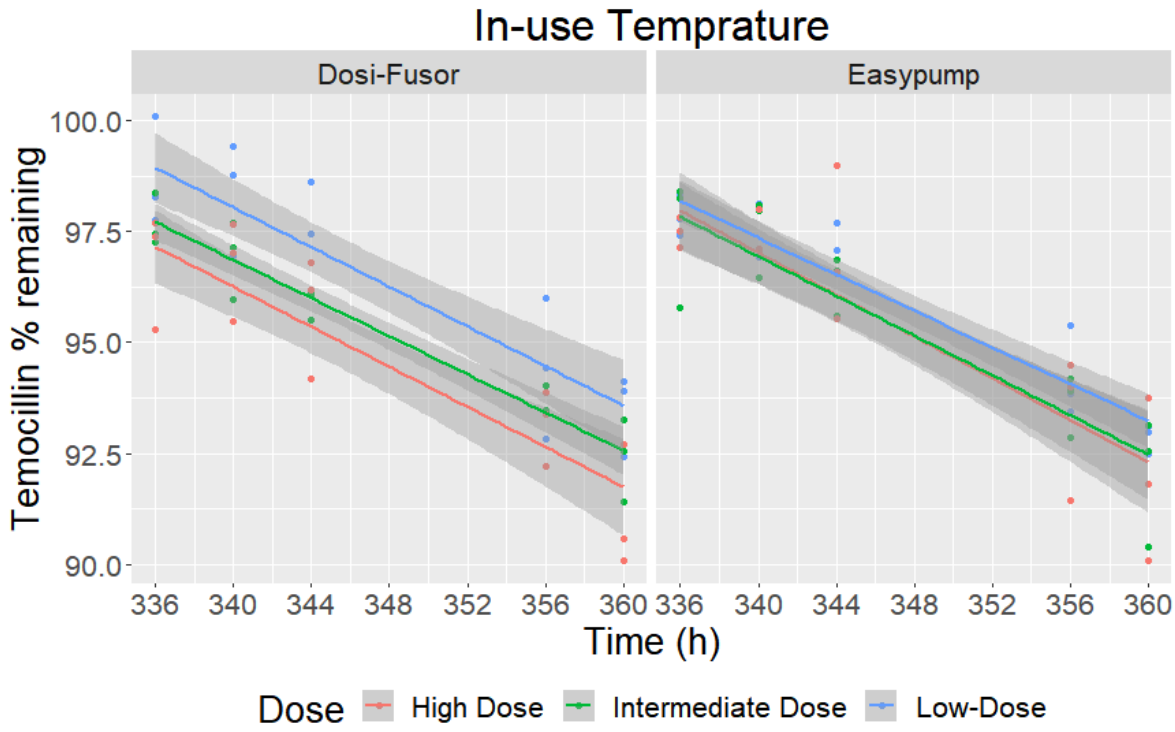

Figure 6. Comparison of percentage of temocillin remaining during 24 hour in-use temperature exposure (32°C) following 14 days of fridge storage, by dose and device type.

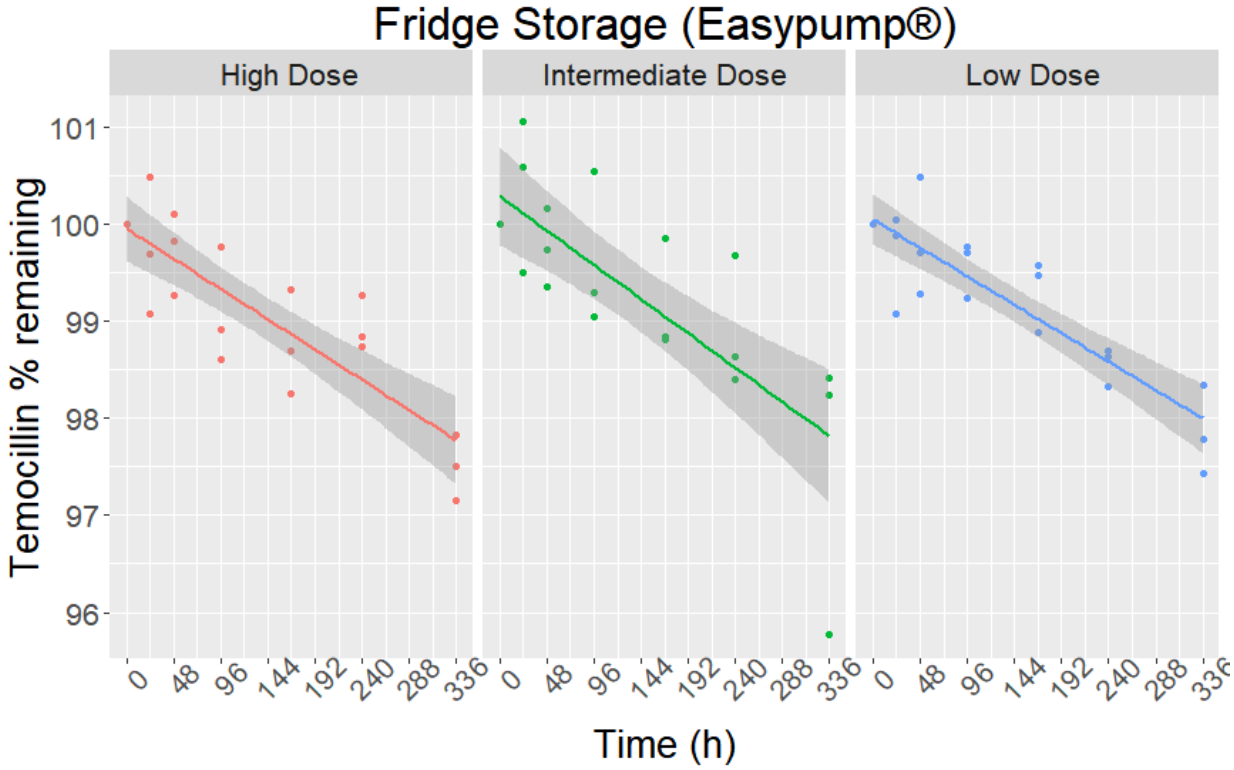

Figure 7. Percentage of temocillin remaining during fridge storage (5°C +/-3°C) for 14 days at high (6000 mg/240 mL), intermediate (4000 mg/240 mL) and low (500 mg/240 mL) dose in Easypump® II LT 270-27- S elastomeric infussion device.

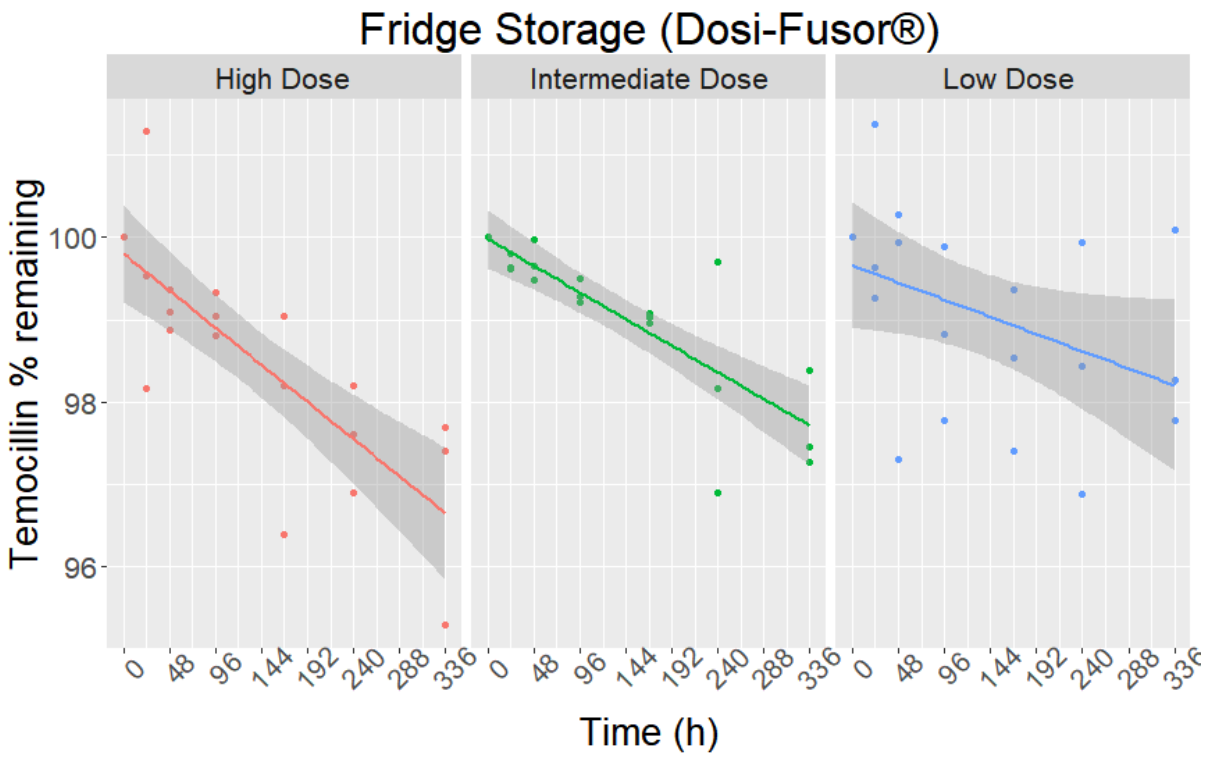

Figure 8. Percentage of temocillin remaining during fridge storage (5°C +/-3°C) for 14 days at high (6000 mg/240 mL), intermediate (4000 mg/240 mL) and low (500 mg/240 mL) dose in Dosi-Fusor® L25915-250D1 elastomeric infusion device.

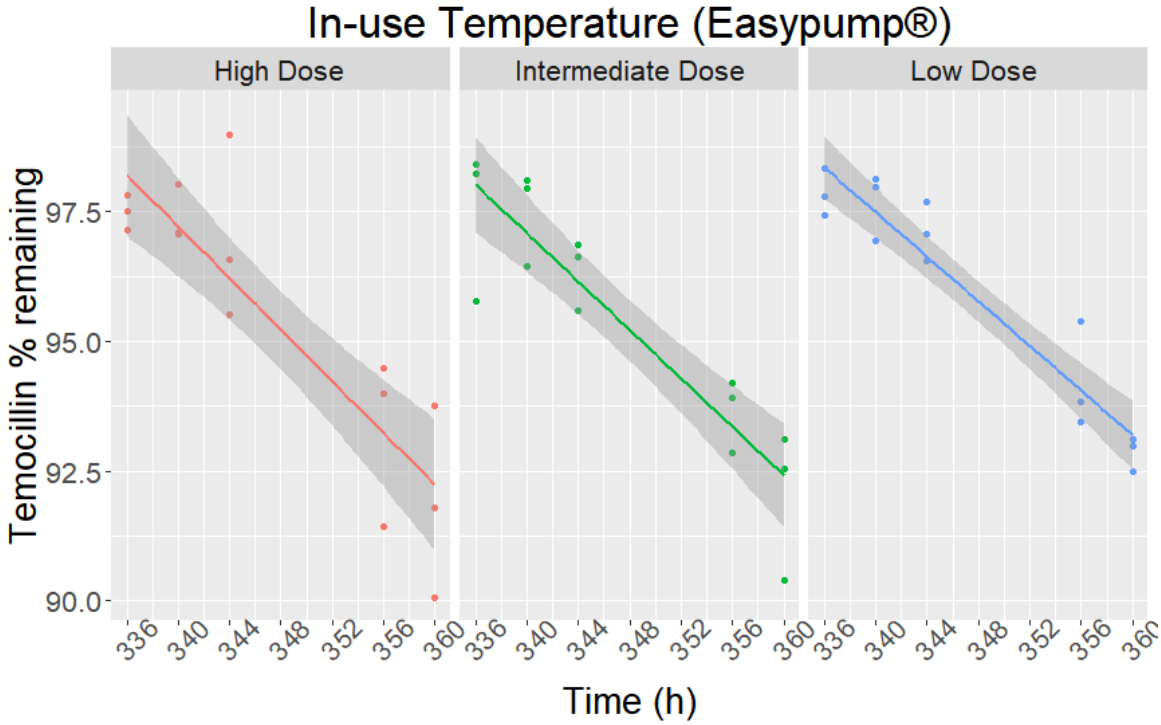

Figure 9. Percentage of temocillin remaining during 24 hour in-use temperature exposure (32°C) following 14 days of fridge storage at high (6000 mg/240 mL), intermediate (4000 mg/240 mL) and low (500 mg/240 mL) dose in Easypump® II LT 270-27-S elastomeric infusion device.

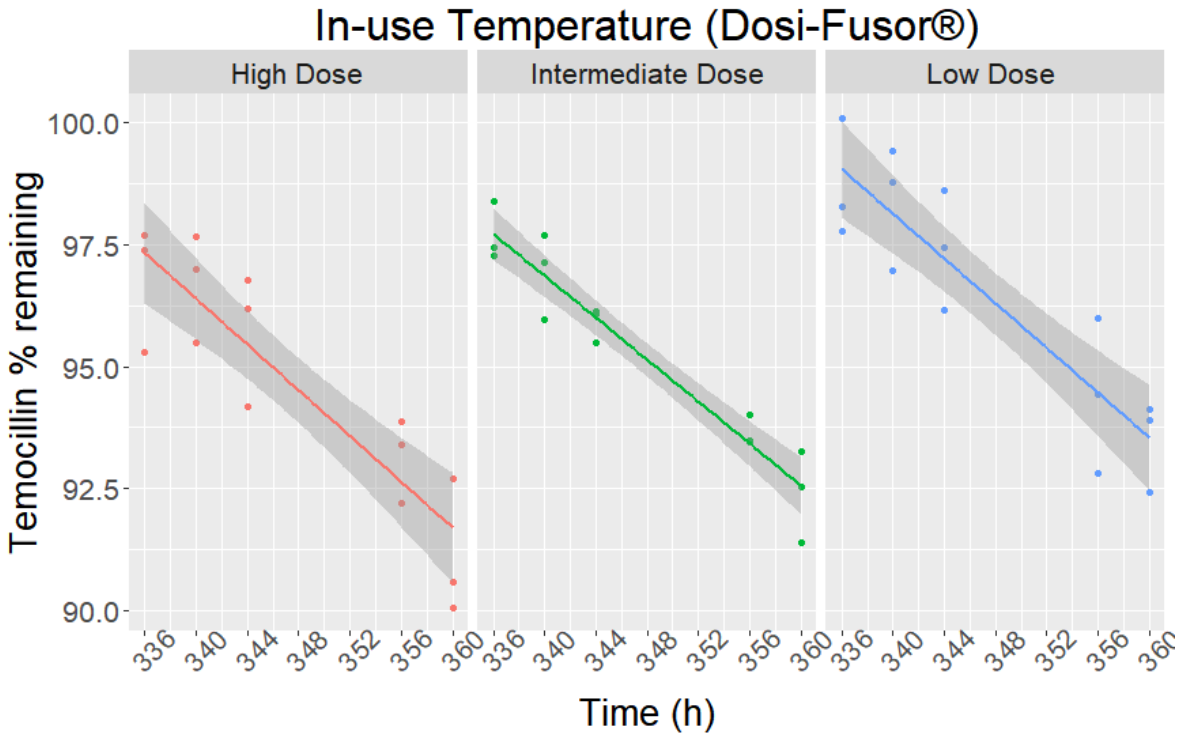

Figure 10. Percentage of temocillin remaining during 24 hour in-use temperature exposure (32°C) following 14 days of fridge storage at high (6000 mg/240 mL), intermediate (4000 mg/240 mL) and low (500 mg/240 mL) dose in Dosi-Fusor® L25915-250D1 elastomeric infusion device.

9.4 Sub-visible particles

All samples were scanned for sub-visible liquid particles analysis by Zetasizer (ZEN300, Malvern Instruments Ltd.). The maximum particle size measured was 5.5 micron. There was no difference in the measured particle sizes either by concentration, device type, or temperature of storage. No trend of increasing particle size was noted during either fridge storage or in-use temperature exposure.

9.5 Degradation products

A semi-quantitative analysis of major temocillin degradation products A, B, C, D and E shown in Figure 1 was performed based on the peak area relative to the temocillin peak area. Summary of the results at each of the low, intermediate, and high dose preparations is given below.

9.5.1 Low dose (500 mg in 240 mL; 2.17 mg/mL)

Table 7 summarises the cumulative amount of major temocillin degradation products as percent ration of temocillin for the Easypump and Dosi-fusor devices at low the dose of 500 mg/240 mL (2.17 mg/mL).

Table 7. Degradation peaks A, B, C, D and E summed area as a percentage of the temocillin peak area

Temperature Condition	Time (h)	% Mean ± SD degradation peak areas by device type	
		Easypump® II LT 270-27- S	Dosi-Fusor® L25915-250D1
		Mean ± SD (%)	Mean ± SD (%)
Fridge storage (5°C +/- 3°C)	0	0 ± 0	0 ± 0
	24	0 ± 0	0 ± 0
	48	0 ± 0	0 ± 0
	96	0.3 ± 0.1	0 ± 0
	168	0.6 ± 0.1	1.3 ± 0.5
	240	1 ± 0.1	1.8 ± 0.6
	336	1.1 ± 0.1	2.1 ± 0.6
In-use temperature (32°C)	340	1.2 ± 0.2	2.2 ± 0.5
	344	1.8 ± 0.1	2.4 ± 0.4
	356	3 ± 0.1	3.8 ± 0.7
	360	3.6 ± 0.3	4.2 ± 0.8

Figure 11 below presents a schematic representation of the cumulative amount of major degradation products A, B, C, D and E relative to the temocillin for the Easypump® II LT 270-27-S and Dosi-Fusor® L25915-250D1 devices filled to 240 mL final volume with the low daily dose of 500 mg temocillin; initial concentration of 2.17 mg/mL.

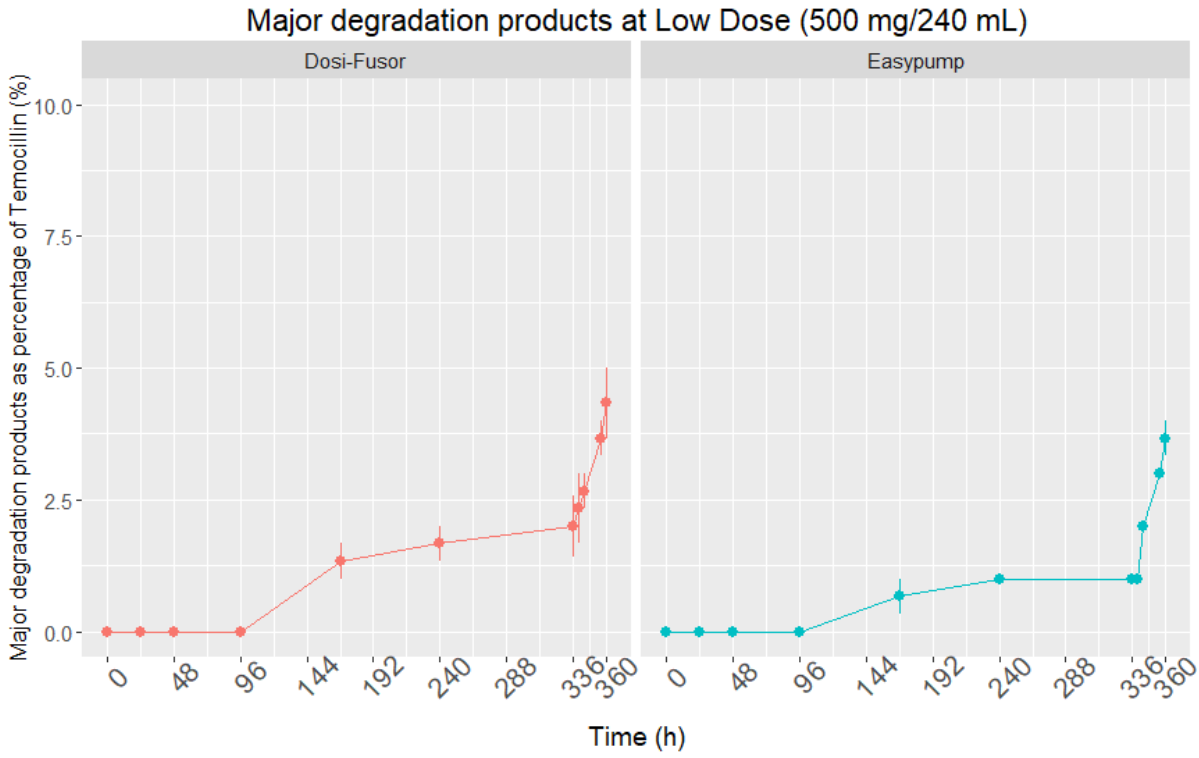

Figure 11. Percentage of major degradation products relative to temocillin during fridge storage (5°C +/-3°C) from time 0 to 336 hour followed by in-use temperature storage at 32°C (at a low dose of 500 mg in 240 mL; 2.17 mg/mL).

9.5.2 Intermediate dose (4000 mg in 240 mL; 16.67 mg/mL)

Table 8. Degradation peaks A, B, C, D and E summed area as a percentage of the temocillin peak area (intermediate dose of 4000 mg in 240 mL).

Temperature Condition	Time (h)	% Mean ± SD degradation peak areas by device type	
		Easypump® II LT 270-27- S	Dosi-Fusor® L25915-250D1
		Mean ± SD (%)	Mean ± SD (%)
Fridge storage (5°C +/- 3°C)	0	0 ± 0	0 ± 0
	24	0.1 ± 0.1	0.1 ± 0.1
	48	0.3 ± 0.1	0.4 ± 0.3
	96	0.7 ± 0.2	0.9 ± 0.1
	168	0.9 ± 0.2	1.1 ± 0.1
	240	1.3 ± 0.3	1.5 ± 0.2
	336	1.6 ± 0.4	1.8 ± 0.4
In-use temperature (32°C)	340	1.8 ± 0.3	2 ± 0.1
	344	2 ± 0.2	2.1 ± 0.3
	356	3 ± 0	2.8 ± 0.1
	360	3.3 ± 0.1	3.4 ± 0.3

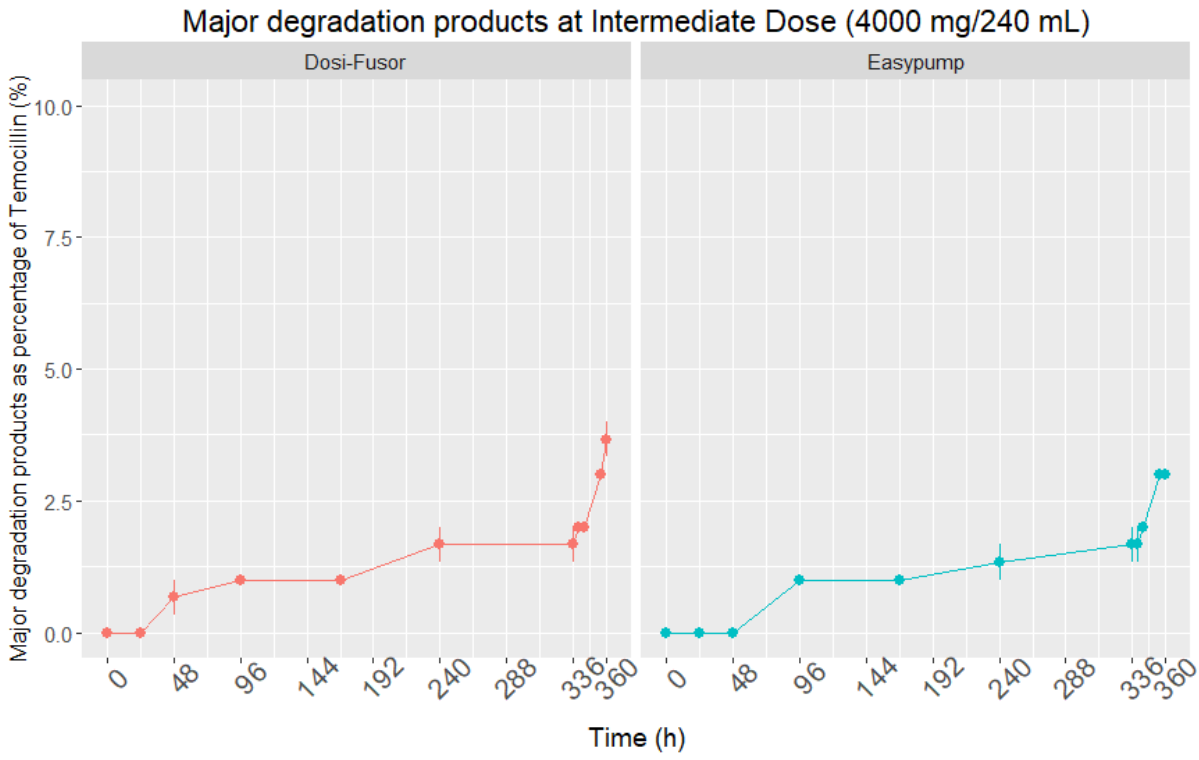

Figure 12. Percentage of major degradation products relative to temocillin during fridge storage (5°C +/-3°C) from time 0 to 336 hour followed by in-use temperature storage at 32°C (at an intermediate dose of 4000 mg in 240 mL; 16.67 mg/mL).

9.5.3 High dose (6000 mg in 240 mL; 25 mg/mL)

Table 9. Degradation peaks A, B, C, D and E summed area as a percentage of the temocillin peak area (high dose of 6000 mg in 240 mL).

Temperature Condition	Time (h)	% Mean ± SD degradation peak areas by device type	
		Easypump® II LT 270-27- S	Dosi-Fusor® L25915-250D1
		Mean ± SD (%)	Mean ± SD (%)
Fridge storage (5°C +/-3°C)	0	0 ± 0	0 ± 0
	24	0 ± 0	0 ± 0
	48	0 ± 0.1	0 ± 0
	96	0.5 ± 0.4	0.3 ± 0.1
	168	1.3 ± 0.7	0.9 ± 0.4
	240	1.6 ± 0.7	1.5 ± 0.3
	336	1.9 ± 0.7	2.1 ± 0.5
In-use temperature (32°C)	340	2 ± 0.7	2 ± 0.3
	344	2.5 ± 0.6	2.5 ± 0.4
	356	3.8 ± 0.5	4.1 ± 0.7
	360	4.3 ± 0.6	4.6 ± 0.6

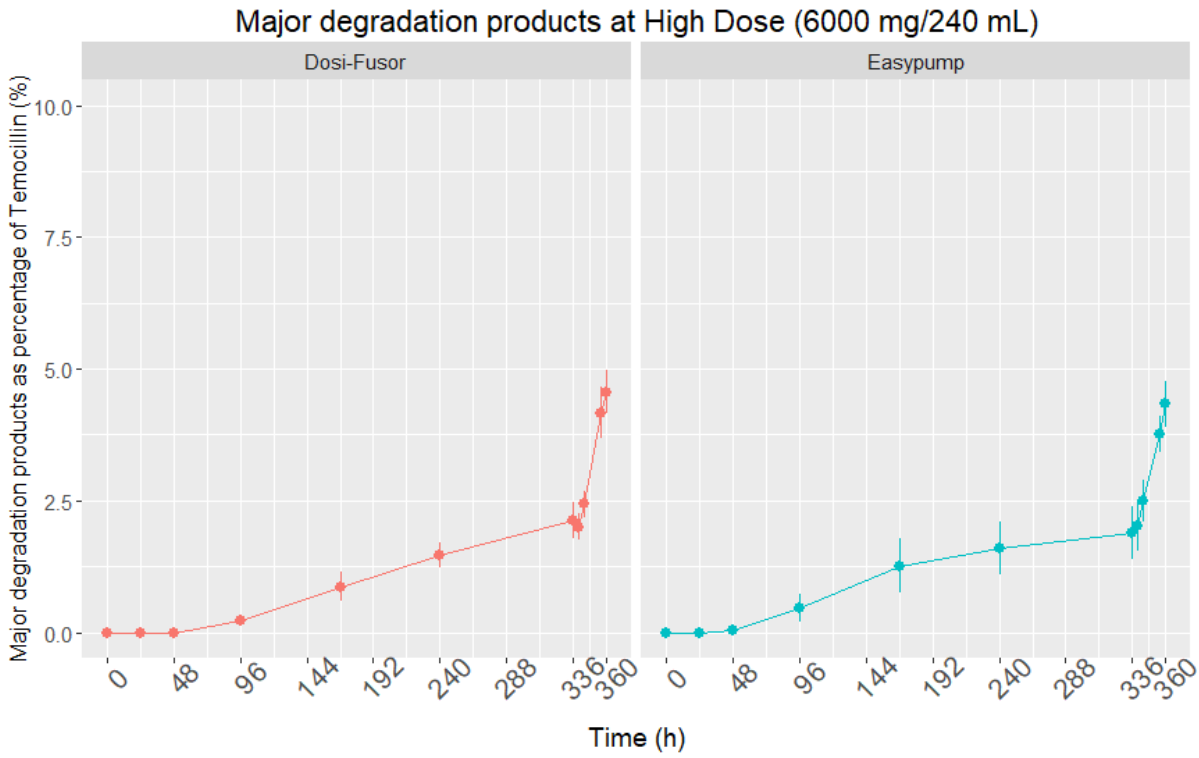

Figure 13. Percentage of major degradation products relative to temocillin during fridge storage (5°C +/-3°C) from time 0 to 336 hour followed by in-use temperature storage at 32°C (at a high dose of 6000 mg in 240 mL; 25 mg/mL).

10. Discussion

This report summarises stability assessment results for temocillin reconstituted in 0.3% citrate buffer (pH7) at low (2.1 mg/mL), intermediate (16.7 mg/mL), and high concentrations (25 mg/mL) in two commercially used elastomeric infusion devices (Easypump® II LT 270-27-S and Dosi-Fusor® L25915-250D1).

During fridge storage for 14 days (336 hours) temocillin exhibited high stability with percentage of temocillin remaining greater than 97% in both devices and at all concentrations (Tables 4-6, Figures 7 & 8). There was no appreciable concentration dependent difference in the percentage of temocillin remaining during 14 days of fridge storage, although slightly higher values were noted at the low concentration for both devices (Figure 5). These results are consistent with a previous study by Rolin *et al*. [27], which showed similar long-term stability of temocillin solution during fridge storage when reconstituted with 5% dextrose and 0.9% sodium chloride at a concentration of 20 mg/L. Considering the 95% acceptance limit, they showed stability was attained for at least 11 days in 5% dextrose and 14 days in 0.9% sodium chloride [27]. Similarly, the study by Carryn *et al*.[23] showed two weeks fridge storage of temocillin in elastomeric infusion pumps achieved stability greater than 97% when reconstituted at 10 or 20 g/L with water for injection.

At the in-use temperature of 32°C, there was a relatively rapid decline in temocillin concentration for both devices, at all concentrations tested (Figures 3-4). At the end of 24 hour in-use temperature exposure, the percentage of temocillin remaining was below the 95% YCD acceptance criteria, but greater than 91% at all concentrations (Tables 4-6). Fitting a linear model to the declining temocillin concentration during in-use temperature, 95% stability limit was maintained for 12 hours for all doses and devices tested except for the high dose of the Dosi-Fusor device which met this criterion for only10 hours of in-use temperature (Figures 9 &10). Although we were unable to find another published study in elastomeric devices with similar exposure to the 32°C in-use temperature, the study Carryn et al. [23] reported >95% stability following two weeks of fridge storage followed by 24 hours of exposure to room temperature in two elastomeric device. Such less than 5% degradation at room temperature has also be reported in an earlier study for up to 48 hours [28], however this does not represent the higher in-use temperature exposures, particular in some hot tropical climates such as those of Australia where in-use temperatures were shown to reach in excess of usual room temperature [29,30]. The study by De Jongh *et al.* [20], although not conducted with YCD testing requirements, evaluated stability of temocillin solution in Milli-Q water at a concentration of 83.4 g/L when exposure to temperatures up to 37°C. The authors reported findings contrary to those observed in the current study with only <2% degradation despite the high temperature exposure for up to 24 hours.

The decline in temocillin concentration during in-use temperature observed in the current study was associated progressively decreasing pH of the solution (Tables 2 & 3). The decrease in pH from baseline following 24 hour in-use temperature exposure was consistently 0.4 to 0.5 units for all concentrations. However, the baseline pH at intermediate and high concentration was 6.84 and 6.81 respectively, despite the use of 0.3% citrate buffer at pH7,

perhaps due to relatively lower buffering capacity against these higher concentrations. The final pH at the end of in-use temperature ranged between 6.26 to 6.62. Generally, temocillin is known to exhibit good stability in mildly acidic or basic aqueous solutions in the pH range of 5 to 9, particularly at low concentrations and therefore, the observed changes in pH are less likely to aggravate further degradation [28].

Five major degradation products were identified from the temocillin sample chromatograms with distinct degradation peaks emerging prior to those of the temocillin diastereomers (Figure 1). The gradual increase in the amount of these degradation products mirrored the progressive decline in temocillin concentration (Figures 11-13). Arithmetically, the summation of the total amount of degradation products measured by peak areas accounts for temocillin lost at the corresponding time during both the fridge storage and in-use temperature exposure.

11. Conclusion

Findings of this study suggest temocillin reconstituted with 0.3% citrate buffer in elastomeric infusion devices is stable at 3-8˚C fridge storage meeting the YCD acceptance criteria of <5 % degradation for up to 14 days. At in-use temperature of 32°C, the YCD acceptance criteria was met for only for 12 hours. However, given the YCD underlines that a 10% loss maybe acceptable for some molecules and the results that <9% degradation was observed at the end of 24 hour in-use temperature exposure, the data support the use of temocillin for 24 hour continuous infusion in elastomeric infusion devices. In jurisdictions where <5% degradation must be strictly followed, twice daily dosing of temocillin maybe considered as an option.

12. References

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