Evaluation of the stability of aciclovir 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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Introduction

Aciclovir is an anti-viral drug introduced in 1982 as a topical agent and later in 1983 for intravenous treatment of herpes virus infections(1). It can be used to treat infections caused by herpes simplex virus (HSV), varicella-zoster virus and Epsten-Barr virus (EB) (2, 3). Structurally, it is a guanine nucleoside analogue and therefore serves as a false substrate in the synthesis of virial DNA effectively blocking the synthesis of viral DNA and the proliferation of the virus(4).

Aciclovir is available in various dosage forms including topical preparation, oral formulations and intravenous solution or powered for injection. Oral Aciclovir has a limited efficacy due to poor bioavailability(5). A prodrug of Aciclovir with improved oral bioavailability, valaciclovir, may be preferably used when increased systemic exposure is necessary to ensure the desired patient outcome(6). Nevertheless, valaciclovir has not been adequately

studied as a substitute to intravenous Aciclovir. Therefore, in some clinical conditions, such as disseminated herpes simplex infections in neonates, prolonged (several weeks) intravenous Aciclovir therapy may be necessary(7). Currently, intravenous Aciclovir remains the gold standard for the treatment of congenital HSV infections in neonates(8). It is also preferred treatment in adult patients with severe disseminated infections(9), which may require prolonged intravenous therapy.

In stable patients that may require or benefit from prolonged intravenous therapy, outpatient parenteral antimicrobial therapy (OPAT) program may be used to effectively deliver Aciclovir therapy. Indeed, Aciclovir has been used in OPAT, particularly for central nervous system (e.g., herpes encephalitis) or disseminated infections of herpes simplex virus and varicella zoster virus (10, 11). However, there is limited data on Aciclovir stability at conditions of OPAT use. Previous data from non-OPAT condition stability studies suggest that it very stable at 5^{o}C and 25^{o}C for up to 37 days without any significant loss of potency, when reconstituted in 5% dextrose or 0.9% sodium chloride solution at a concentration of 5 mg/mL(12). However, the authors observed white icy precipitation of Aciclovir at 5^{o}C that re-dissolved when the temperature was brought to 25^{o}C. A later study (13) tested Aciclovir stability at low (1mg/mL), intermediate (7mg/mL) and (10mg/mL) high concentrations using the same solvents (5 % dextrose or 0.9% sodium chloride) and similar temperatures of 4^{o}C and 23^{o}C. No precipitate was noted for the low concentration when stored at 4^{o}C, however at the intermediate and higher concentrations tested, precipitation was noted as previously reported. Similarly, at 5mg/mL Aciclovir solution in 0.9% sodium chloride another study did not observe any precipitation when was stored at 4^{o}C and demonstrated stability at least for 21 days of refrigeration (14). A recent study with similar conditions reported an extended fridge stability at 5mg/mL concentrations of over 63 days (15).

In OPAT use, antimicrobials are often stored in fridge (or ambient temperature) for 7 to 14 days followed by 24-hour exposure to in-use conditions. Previous studies have shown that in-use temperatures of OPAT antimicrobial solution in infusion pumps exceeds the usual ambient room temperature of 25^{o}C; generally 32^{o}C is considered the maximum in-use temperature reached in most of the world although in some hot climate zones this may reach up to 34^{o}C (16). Unfortunately, no stability data exist for Aciclovir at these in-use temperature conditions and including the range of doses of clinical interest, as prescribed in contemporary guidelines such as the UK NHS Yellow Cover Document(17).

The aim of this study was, therefore, to evaluate the stability of Aciclovir in elastomeric infusion devices used for Outpatient Parenteral Antimicrobial Therapy in accordance with the requirement of the UK NHS Yellow Cover Document.

Assay Method Development and Validation

Background

The stability indicating assay method by Huidobro, et al.(18) separates Aciclovir from its degradation product guanine, as well as 6 known impurities.

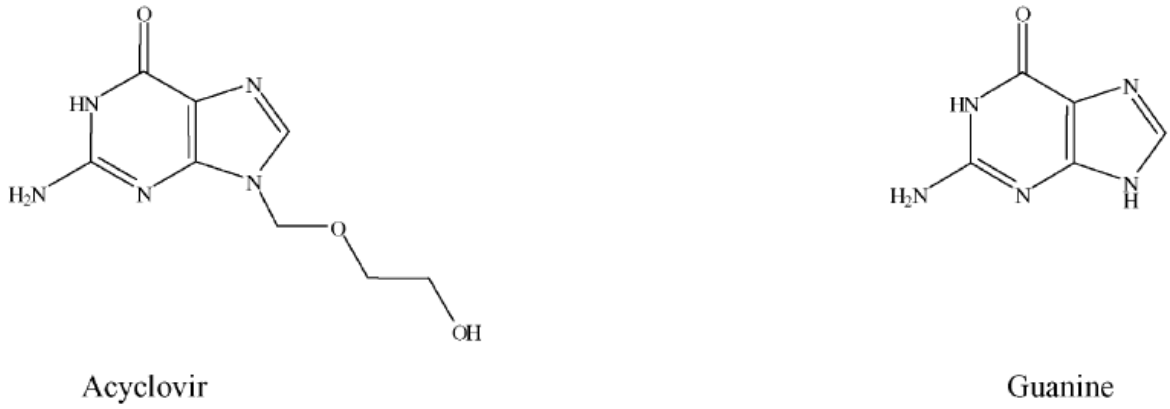

Figure 1: Aciclovir and Guanine (from Huidobro, 2005)

Guanine is insoluble in water. Darvishzad, et al.(19) quantified its aqueous solubility at various pHs at 25°C, concluding 25.4 µM was the limit of solubility at neutral pH.

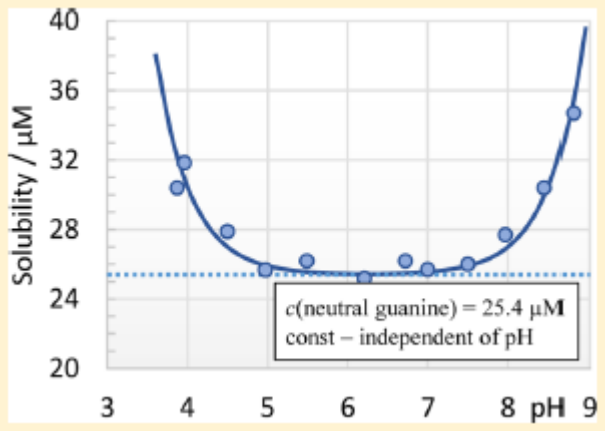

Figure 2: Solubility of guanine (from Darvishzad, 2018)

The Test concentrations for the stability experiment are:

- Low: 200mg/240mL = 833 mg/L = 3699 µM
- High: 4500mg/240mL = 18,750 mg/L = 83,256 µM

The solubility limit of guanine would be reached with the conversion of 0.69% of Aciclovir at the low concentration and 0.03% of Aciclovir at the high concentration.

Assay method

Aciclovir for calibrators and QCs was DBL Aciclovir Intravenous Infusion Batch: H161213AA (Hospira Australia, Mulgrave, Australia), the same batch used to generate the test samples. Guanine (used for degradation identification) was sourced from Sigma Aldrich (St Louis, USA). Water used was Milli-Q.

Calibrators were prepared by dilution of the Aciclovir pharmaceutical product with water to concentrations of 400, 600, 700, 800, 900 and 1000 mg/L. QCs were prepared by dilution of the Aciclovir pharmaceutical product with water to concentrations of 833 and 18,750 mg/L. Calibrators were stored in separate aliquots at -80°C until required.

The chromatography was adapted from the stability indicating method of Malabagal, et al.(20), using an Acquity UPLC BEH C18 (1.8 µm) 2.1 x 30 mm analytical column (Waters, Milford, USA) as stationary phase. Mobile phase was 97% 25 mM phosphate buffer at pH 3.0 with 3% acetonitrile delivered isocratically at 0.4 mL/min with a 2-minute run time.

Calibrators, QCs and test samples were thawed at room temperature then vortex mixed. Calibrators and low concentration (833 mg/L) QCs and samples were centrifuged then directly injected. Samples and QCs at the high concentration were centrifuged and then diluted with a dilution factor of 22.5 (40 µL of sample combined with 860 µL of water) prior to injection.

Assay Performance

Calibrators from 400 to 1000 mg/L (n=6 levels) were used to create the calibration curve, covering a range of 48% to 120% of the nominal test concentration. Linearity of the method was established from 3 separate calibration lines: slopes were 4434, 4504 and 4468; intercepts were -95178, -4504 and -90900; correlation coefficients (r^2) were 0.99992, 0.99881 and 0.99934, with all calibrators being within 0.3%, 1.6% and 1.3% of nominal, respectively.

Precision (%RSD) of the assay, demonstrated by replicate analysis (n=9) of QCs, was 1.2% at 833 mg/L and 1.2% at 18,750 mg/L. Accuracy (% Bias) from the same QCs was -0.2% (833 mg/L) and -1.1% (18,750 mg/L).

Specificity: Aciclovir gave a peak at retention time 0.515 min. Guanine eluted with baseline separation prior to Aciclovir. Huidobro reported impurities were all more strongly retained than Aciclovir, so a secondary chromatographic method that included a gradient of acetonitrile from 3% to 20% was used to search for the impurities but none were found.

Results

Colour, clarity, and precipitation

During 14 days of room temperature storage, aciclovir solution in both the Easypump® II LT 270-27- S and Accufuser® VAWC0100L elastomeric infusion pumps was clear and colourless with no visible precipitation in samples collected or within the body of the infusers. However, from day 7 onwards small crystals were noted at the tip of the sampling tube for the high concentration while the body of the infuser was clear.

During the subsequent exposure to in-use temperature of 32°C, at the low concentration (200 mg/240 mL) the solution remained clear, colourless and no visible precipitation was noted. However, a massive white precipitation was noted for the high concentration (4500 mg/240 mL), Figure 3.

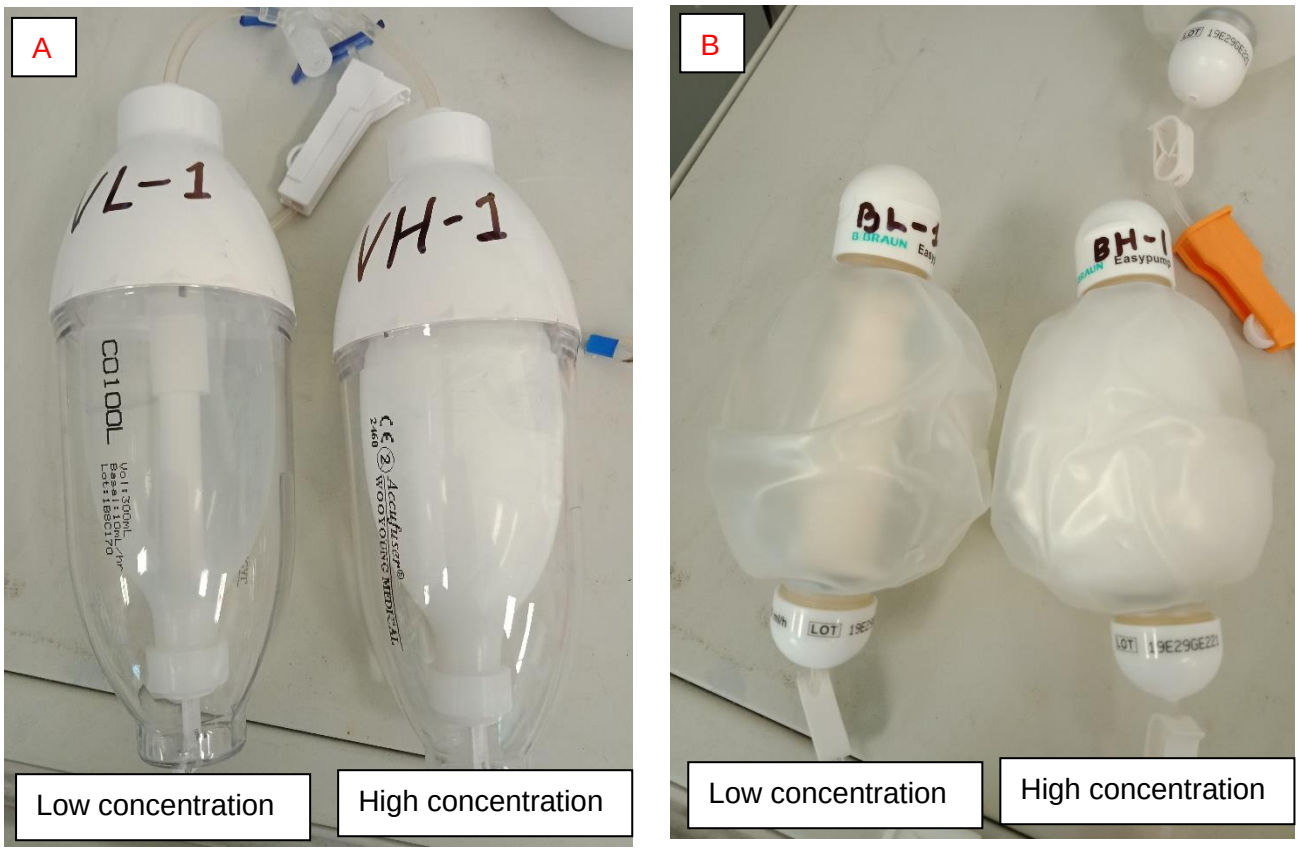

Figure 3. Visible precipitation of white substance noted at 4 hours of storage at 32°C, for the high concentration of aciclovir in Accufuser® (A) and Easypump® II (B) infusion devices. The device on the left side in each panel contained the low concentration (200 mg/240 mL) and the device on the right side contained the high concentration (4500 mg/ 240 mL).

Analysis of Precipitant

A sample of the recovered precipitant paste (0.11 g) was dissolved in 3 mL of 0.1 M NaOH. Injection of the solution revealed a massive (off-scale) Aciclovir peak. For comparison, a solution of guanine in 0.1 M NaOH was injected producing a peak at its earlier retention time. The confirms the precipitate to be Aciclovir.

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 5.6 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 room temperature storage or in-use temperature exposure.

pH

Table 1. Observed change in pH of aciclovir solution in Accufuser® VAWC0100L elastomeric infusion device during storage at room temperature and subsequent exposure to in-use temperature of 32°C

Temperature condition	Time	Observed mean ± SD pH and change in mean pH from baseline by concentration			
		Low Concentration		High Concentration	
		Observed pH	D pH	Observed pH	D pH
Room Temperature	0	10.24 ± 0.01	0.00	11.25 ± 0.01	0.00
	12	10.3 ± 0.01	-0.05	11.25 ± 0.01	0.00
	24	10.27 ± 0.03	-0.03	11.27 ± 0.01	-0.01
	48	10.17 ± 0.03	0.07	11.22 ± 0.02	0.03
	96	10.02 ± 0.06	0.23	11.25 ± 0.05	0.01
	120	9.85 ± 0.07	0.39	11.15 ± 0.04	0.11
	168	9.67 ± 0.09	0.57	11.04 ± 0.08	0.21
	240	9.47 ± 0.11	0.77	10.94 ± 0.11	0.31
	336	9.26 ± 0.15	0.99	10.88 ± 0.13	0.37
In-use temperature (32 °C)	344	7.43 ± 0.24	2.81	10.44 ± 0.05	0.82
	348	6.91 ± 0.02	3.33	10.28 ± 0.09	0.98
	360	6.86 ± 0.04	3.38	10.06 ± 0.21	1.20

Table 2. Observed change in pH of aciclovir solution in Easypump® II LT 270-27- S elastomeric infusion device during storage at room temperature and subsequent exposure to in-use temperature of 32°C

Temperature condition	Time	Observed mean ± SD pH and change in mean pH from baseline by concentration

		Low Concentration		High Concentration	
		Observed pH	D pH	Observed pH	D pH
Room Temperature	0	10.11 ± 0.01	0.00	11.13 ± 0.01	0.00
	12	10.23 ± 0.04	-0.12	11.26 ± 0.02	-0.13
	24	10.22 ± 0.02	-0.11	11.27 ± 0.01	-0.14
	48	10.08 ± 0.04	0.03	11.22 ± 0.01	-0.09
	96	9.88 ± 0.02	0.23	11.22 ± 0.01	-0.09
	120	9.71 ± 0.02	0.40	11.06 ± 0.02	0.07
	168	9.5 ± 0.04	0.61	10.92 ± 0.02	0.22
	240	9.26 ± 0.06	0.85	10.82 ± 0.02	0.32
	336	9 ± 0.04	1.11	10.74 ± 0.01	0.39
In-use temperature (32 °C)	344	7.28 ± 0.4	2.83	10.35 ± 0.02	0.78
	348	6.82 ± 0.02	3.29	10.18 ± 0.04	0.95
	360	6.85 ± 0.05	3.26	9.82 ± 0.08	1.31

Aciclovir percent remaining

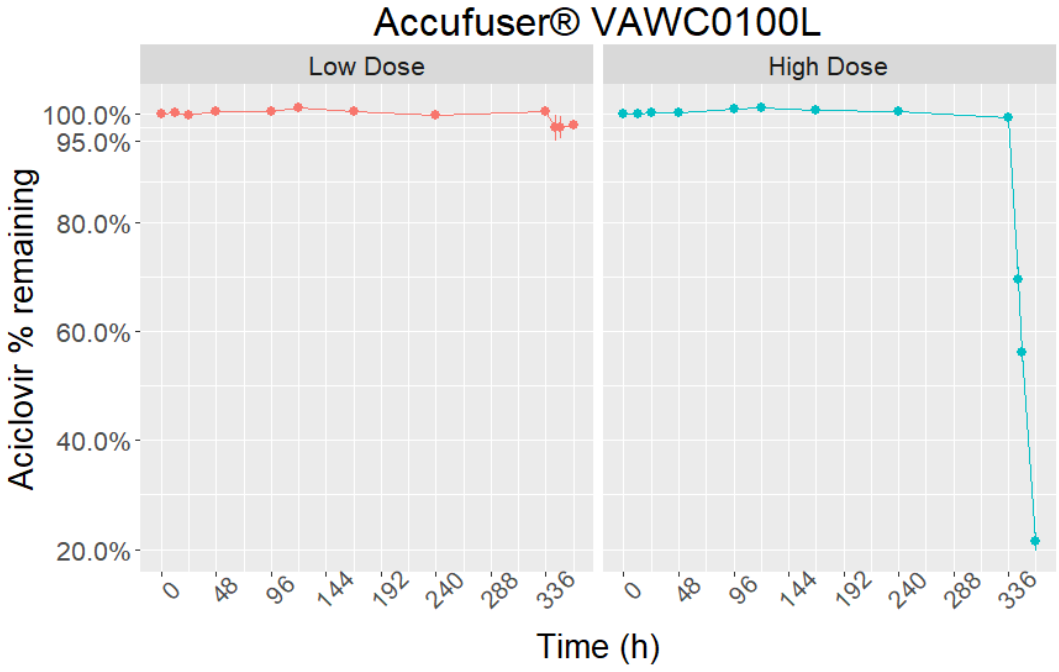

Figure 4. Percentage of aciclovir remaining in Accufuser® VAWC0100L elastomeric infusion device during storage at room temperature for 14 days (336 hours) followed by 24 hour in-use temperature exposure at 32°C. Low dose= 200 mg/ 240 mL; High dose= 4500 mg/ 240 mL)

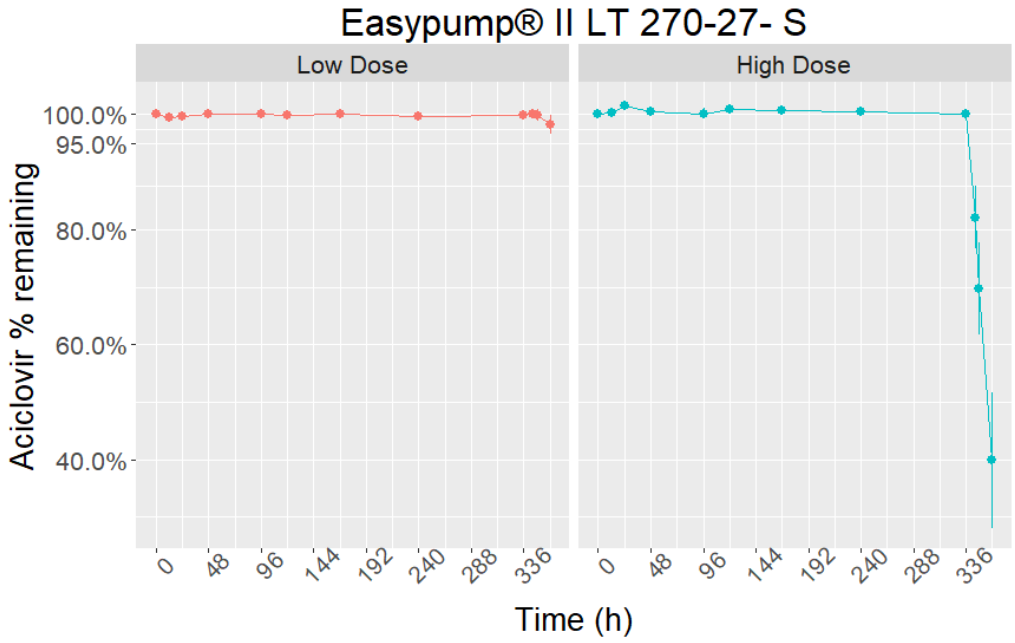

Figure 5. Percentage of aciclovir remaining in Easypump® II LT 270-27- S elastomeric infusion device during storage at room temperature for 14 days (336 hours) followed by 24 hour in-use temperature exposure at 32 °C. Low dose= 200 mg/ 240 mL; High dose= 4500 mg/ 240 mL)

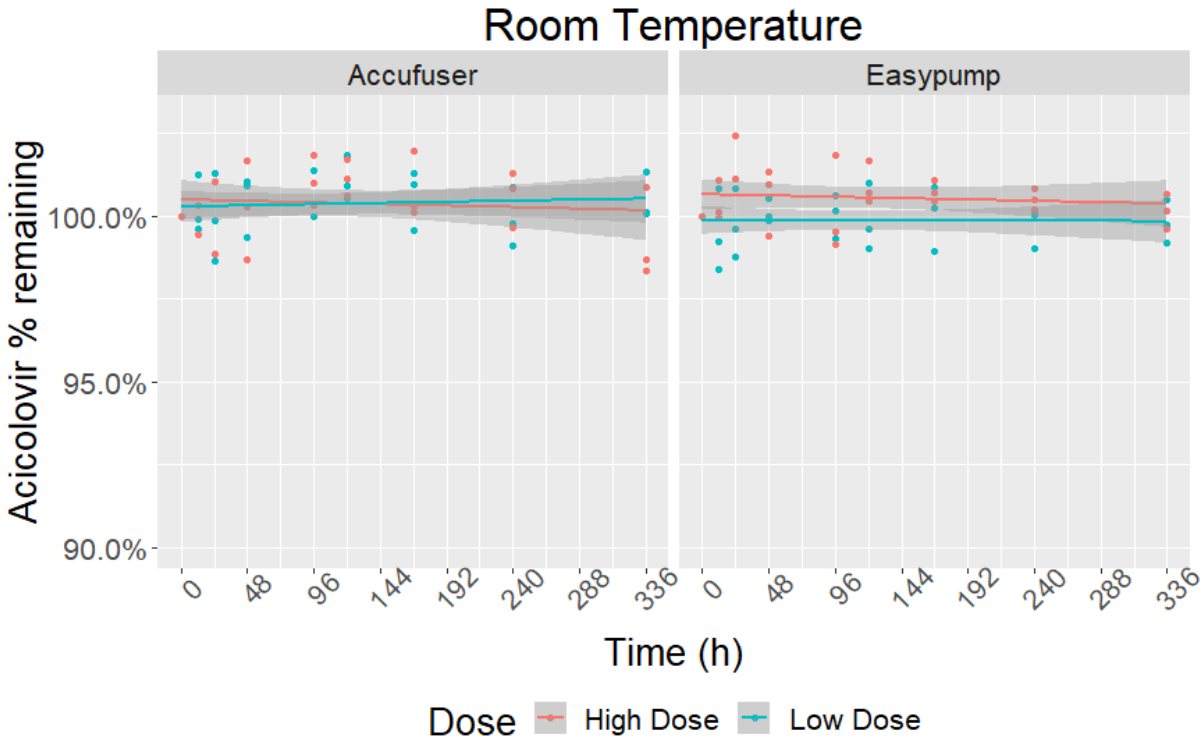

Figure 6. Percentage of aciclovir remaining during room temperature storage for 14 days (336 hours) by device and dose. Low dose= 200 mg/ 240 mL; High dose= 4500 mg/ 240 mL)

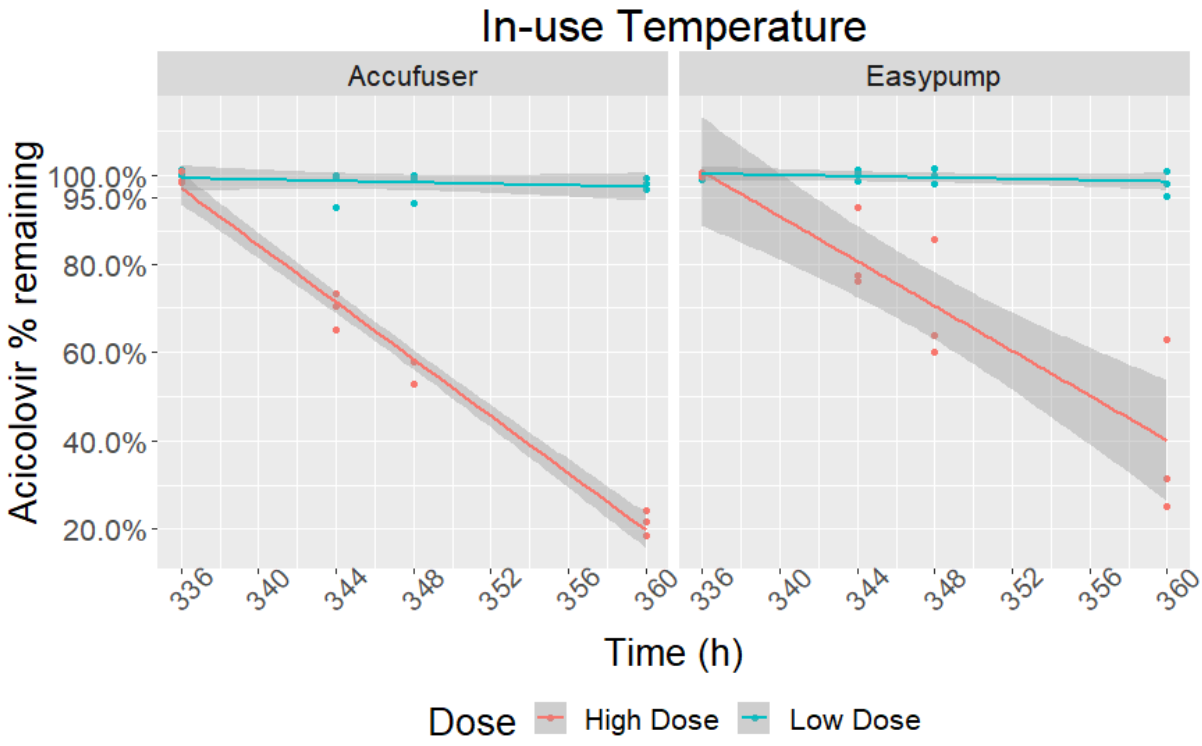

Figure 7. Percentage of aciclovir remaining during exposure to in-use temperature of 32°C following 14 days (336 hours) room temperature storage, by device and dose. Low dose= 200 mg/ 240 mL; High dose= 4500 mg/ 240 mL.

Discussion

In this study, the stability of Aciclovir in two elastomeric infusion pumps was tested during 14 days of room temperature storage followed by 24 hours exposure to in-use temperature of 32°C, at a low (833 mg/L) and high (18,750 mg/L) concentration.

During room temperature storage, Aciclovir remained in solution with no sign of precipitation within the body of the infusers. Aciclovir is known to from a white icy precipitate at fridge storage temperatures, which re-dissolves when brought to room temperature 25°C at relatively low concentrations (12). We noticed a massive insoluble precipitation at high concentration tested when exposed to in-use temperature of 32°C (Figure 3). Our observation is consistent with that of Zhang and colleagues(13) who reported a persistent micro precipitate that did not dissolve when Aciclovir solution in 0.5% dextrose or 0.9% sodium chloride was stored at 4°C followed by 2 days at 23°C protected from light

as in the current study. This insoluble precipitation was observed at concentrations about half of the high concentration used in this study (7000 and 10000 mg/L).

Precipitation of Aciclovir is not an *in vitro* problem only; it can occur *in vivo* in renal tubules if the maximum solubility of free Aciclovir (2.5 mg/mL at 37^{o}C in water) is exceeded. Intravenous infusion of high concentration therefore risks acute renal failure due to tubular damage and must be accompanied by adequate hydration(21, 22). Indeed, the most common mechanism of Aciclovir-induced acute kidney injury is due to crystal obstruction(23). The use of high concentration (dose) of Aciclovir in OPAT setting requires a careful safety consideration. Although the slow, continuous infusion in OPAT is advantageous in minimising risk of precipitation when compared to the traditional 1-2 hours of infusion for in-patient's Aciclovir intermittent therapy, maintaining and monitoring fluid balance in outpatient settings may not be practical. Thus, patients should be educated on the importance of maintaining adequate hydration.

The massive decline in Aciclovir concentration during exposure to in-use temperature parallels the massive precipitation observed (Figure 7). Samples of the precipitate were tested and confirmed to be Aciclovir by HPLC analysis (data not shown here) suggesting that the decline in Aciclovir concentration is mainly due to precipitation and not decomposition. This observation is in agreement with the Zhang *et al.* study (13) which attributed reduction in Aciclovir concentration to precipitation but not to decomposition.

Conclusion

At the low dose of 200 mg/ 240 mL (equivalent to 833 mg/L) aciclovir solution is stable in Accufusor and Easypump elastomeric infusion pumps for 14 days at room temperature and 24 hours of 32°C in-use temperature exposure. At this low concentration, it meets the requirements of the Yellow Cover Document with 95% remaining as a limit of acceptance. However, at the higher dose tested, 4500 mg/ 240 mL (equivalent to 18,750 mg/L), although it is stable at room temperature, it massively precipitates during exposure to in-use temperature of 32°C.

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