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Stability Test of Ampicillin Sodium Solutions in the Accufuser® Elastomeric Infusion Device Using HPLC-UV Method

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ABSTRACT

The stabilities of two kinds of solutions (30 mg/mL) of Ampicillin sodium in 0.9% NaCl in water (NS, normal saline) and in sterile water (SW) in the intravenous elastomeric infusion device (Accufuser®) were evaluated based on recommended solutions and storage periods. The injectable NS- and SW-Ampicillin solutions in the Accufuser® device were stored and evaluated at controlled temperature (room temperature, 25˚C ± 2˚C and cold temperature, 4˚C ± 2˚C) during 7 days. Effects of the periods of storage (from 0 to 7 days) and the temperatures of storage (RT and CT) on the physicochemical appearances and concentrations of active compounds were determined. The visual clarity, pH, and concentrations of Ampicillin were determined by stability-indicating high-performance liquid chromatography (HPLC)-ultraviolet (UV) detection. The results showed that the amount of Ampicillin in studied solutions gradually decreased with time. The Ampicillin in NS, which was stored in CT, was relatively stable, retaining 94% of its original amount up to 7 days. The solution that showed least stability was Ampicillin in SW, which was stored in RT, retaining 80% of its original amount. Generally, solutions that were stored in CT were more stable than the solutions that were stored in RT. No significant changes in physical appearance or color of the solutions were observed during the study. Particles were not detected in any solution samples. In summary, two kinds of solutions of Ampicillin sodium, in NS and SW, showed different chemical stabilities with time in intravenous infusion device without any significant physical changes and retained about 94% vs 89% and 83% vs 80% of initial concentrations after 7 days in CT and RT, respectively. We suggest that 30 mg/mL of Ampicillin sodium in NS solution in an Accufuser® infusion device which is stored in CT can be applicable for 7 days in clinical situations.

Keywords: Ampicillin Sodium; Intravenous Elastomeric Infusion Device (Accufuser®); HPLC-UV Method; Solution Stability

1. Introduction

The disposable silicon balloon infusion device (Accufuser®, Woo Young Medical Co. LTD., Seoul, South Korea) is a well-established simplified silicon-based elastomeric system for the administration of antibiotics and other drugs or nutrients that are suitable for patients as well as healthcare providers. An increasing number of patients are being treated as outpatients, and for them, drugs are often infused using portable pumps or infusion devices outside the hospital. To be suitable for self-administration by home-based patient, the antibiotic should be stable in the peritoneal dialysis solution for a number of days under home storage conditions [1]. Therefore, it is necessary to determine the physical and chemical stabilities of the admixtures in the infusion system before they appear in the clinical settings [2-8]. The purpose of this study is to provide such information with commonly used antibiotic solutions, Ampicillin sodium, into Accufuser® elastomeric infusion device under recommended storage conditions. The physical and chemical stabilities of Ampicillin sodium (**Figure 1**) solutions (30 mg/mL, normal

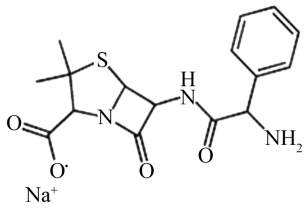

Figure 1. Chemical structure of ampicillin sodium ($C_{16}H_{19}N_3O_4S$·Na).

saline, NS and sterile water, SW) packed in sterile Accufuser® device were evaluated, in which each samples were stored and evaluated at appropriate intervals up to 7 days under different storage conditions (room temperature, RT, 25˚C ± 2˚C and cold temperature, CT, 4˚C ± 2˚C). The study was done with Ampicillin sodium solutions which were made with NS and SW since these are the most available infusion solutions for Ampicillin sodium administrations in clinical situations [1,6,9].

2. Materials and Methods

2.1. Materials

Ampicillin Sodium was purchased from Fluka Co., USA. Normal saline (NS, 0.9% NaCl in water) and sterile water (SW, injectable distilled water) were purchased from Choongwae Pharmaceutical Co., South Korea. Disposable Silicon Balloon Infuser (Accufuser®, **Figure 2**) was obtained from Woo Young Medical Co., LTD, South Korea. Acetonitrile and potassium phosphate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and other chemicals for HPLC analysis were all HPLC-grade and were prepared immediately before use. Millipore's Milli-Q system (MA, USA) was used throughout the analysis.

2.2. Preparation and Sampling of Solution

To prepare the samples, the appropriate amounts of Ampicillin sodium were added to the portion of the infusion solution and were brought to a final volume of 20mL with NS and SW. The test solutions were packaged in sterile Accufuser® system for testing. All manipulations were performed in a biological safety cabinet. The nominal concentration of Ampicillin sodium solution for the sample used in this testing was 30 mg/mL. Triplicate test solutions under each storage conditions were prepared. The test solutions were stored at RT (25˚C ± 2˚C) and CT (4˚C ± 2˚C). Aliquots were removed from each test solution initially and at the intervals of each day during 7 days at 25˚C ± 2˚C and 4˚C ± 2˚C conditions and the concentrations of Ampicillin sodium were quantified by the HPLC-UV method (**Table 1**).

2.3. Methods

The physical stabilities of the Ampicillin infusion solutions were assessed by visual examination and HPLC analysis. Visual examinations were performed in normal diffuse fluorescent room light with unaided eye and high-intensity monodirectional light. The pH of the solutions was measured by a stainless electrode pH meter

Table1. Study designs for stability testing of ampicillin sodium solutions (30 mg/mL) in Accufuser® system.

˚C	Solutions \ Day	0	1	2	3	4	5	6	7
RT	NS	*o	o	o	o	o	o	o	o
	SW	o	o	o	o	o	o	o	o
CT	NS	o	o	o	o	o	o	o	o
	SW	o	o	o	o	o	o	o	o

*o: processed sample; RT: room temperature; CT: cold temperature; NS: normal saline; SW: sterile water.

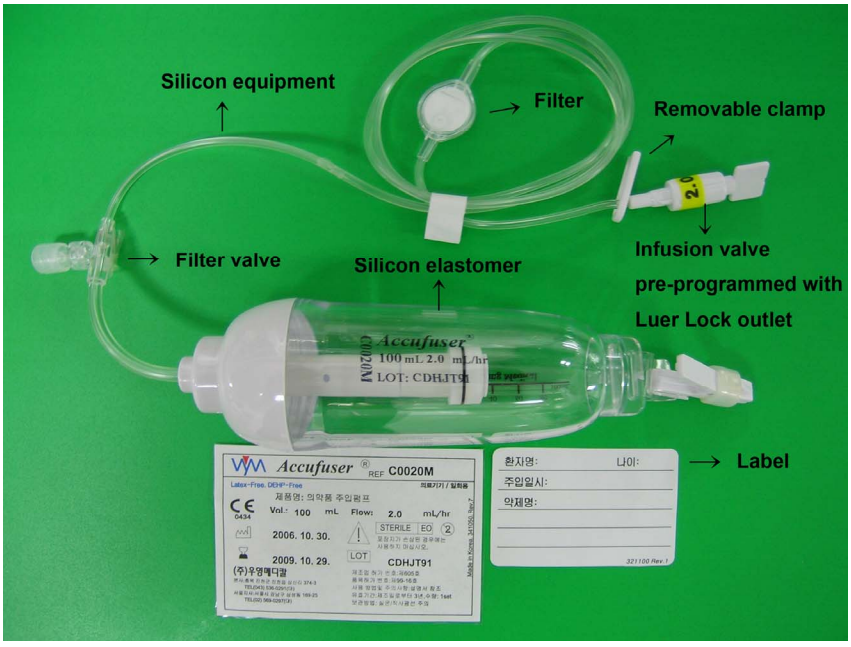

Figure 2. Disposable silicon balloon infusion system (Accufuser®, Woo Young Medical Co., LTD, South Korea).

(Thermo Scientific Co., MA, USA). The drug concentrations were determined using a stability-indicating HPLC assay method based on several references [10,11]. The HPLC system [10,12-14] consisted of an isocratic solvent delivery pump (Model 515, Waters Scientific Co. USA) which pumped a mixture (v/v, 60/40, pH 3.0) of acetonitrile (ACN, Sigma Co. USA) in 0.05M potassium phosphate (Sigma Co. USA) through a Capsell Pak C18 UG120 (4.6 × 250 mm, 5 µm) column at 1.0 mL/min. The ratio of ACN to 0.05 M potassium phosphate (60/40) was held constant during the chromatographic run. The samples of 2.0 µL were injected into the HPLC system using an autosampler (Nanospace SI-2, Shiseido Co., Japan). The effluent from the column was monitored with a variable wavelength ultraviolet detector (Nanospace SI-1, Shiseido Co., Japan) at 220 nm. The integration of the chromatograms was performed by dsCHROM® software (Do-Nam Instrumental Co., Seoul, Korea). The method was validated for linearity, precision (inter-day and intra-day), accuracy and selectivity. The standard plot was constructed for Ampicillin in the range of 0 - 3.33 mg/mL. The experiment was repeated five times on the same day and additionally on four consecutive days to determine inter- and intra-day precisions [12]. Assays of control solutions from Ampicillin sodium solutions (3mg/mL) were undertaken to calculate the intra-day and inter-day variations using external standard method. Linearity was evaluated by serial dilutions of Ampicillin sodium solutions with NS and SW for analysis. Linear regression analysis of peak area and drug concentration yielded a good correlation coefficient > 0.99 with range from 0 to 3.33 mg/mL (**Figure 3**). The stability of Ampicillin sodium infusion solutions was determined in disposable infusion device (Accufuser®) during 7 days of storage under RT and CT conditions [5]. Periodically, samples were evaluated for appearances, visible particles, pH and chromatographic analysis. We analyzed the concentration of Ampicillin sodium in two solutions at each day during 7 days after the preparation of solutions by HPLC-UV method [10,12,13]. On each day, 1.0 mL of samples of Ampicillin sodium with a nominal concentration were drawn from Accufuser® infusion device for chromatographic analysis and 2.0 µL were directly injected into HPLC-UV system. The three aliquots of each solution were processed. Statistical analysis was performed using one-way ANOVA with the level of significance set at 0.05 (PCS, version 4.0, Springer-Verlag, New York, USA).

3. Results

There were no significant changes in physical appearances, odors, or clarities of the solutions. The color of the samples was transparent, with no color changes. Particles were not detected in any samples. The pH of NS and SW solutions slightly decreased from 9.20 to 8.49 and from 9.47 to 8.72 in the CT, but significantly ($p < 0.05$) decreased from 9.30 to 6.78 and 9.10 to 7.10 in the RT during 7 days (**Table 2**). The linearity of calibration curves of Ampicillin Sodium was established which showed good linearity over the range concentration of 0 - 3.33 mg/mL ($r^2 = 0.999$, **Figure 3**).

Table 3 lists the relative standard deviation (R.S.D.) data obtained from the analysis of the samples on the same day (n = 5) and on consecutive days (n = 5). The R.S.D values were <1.07 % and <0.92 % for intra-day and inter-day results, respectively, meaning that the method was sufficiently precise. The typical HPLC chromatogram of Ampicillin sodium (200 µg/mL) is shown in **Figure 4**.

The retention time for Ampicillin sodium was about

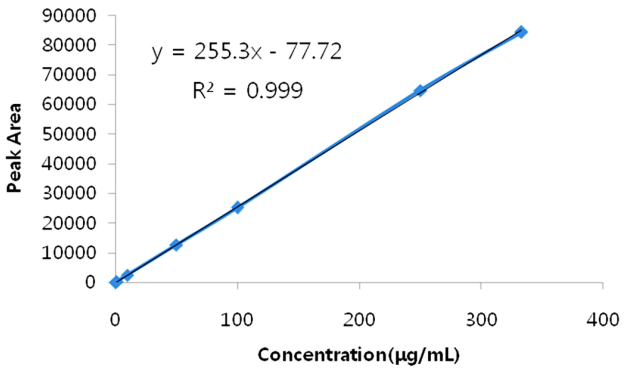

Figure 3. Calibration curve for the determination of ampicillin sodium concentrations (0 - 3.33 mg/mL) in study solutions.

Table 2. The change of pH (Mean ± SD, n = 5) of ampicillin sodium solutions according to the time of storage.

˚C	Time (day) / Solution	0	1	2	3	4	5	6	7
RT (25˚C ± 2˚C)	NS	9.30 ± 0.05	8.71 ± 0.04	8.52 ± 0.00	8.19 ± 0.01	7.81 ± 0.01	7.73 ± 0.04	7.20 ± 0.03	6.78* ± 0.02
	SW	9.10 ± 0.01	8.65 ± 0.01	8.50 ± 0.02	8.14 ± 0.03	8.10 ± 0.01	7.90 ± 0.02	7.54 ± 0.01	7.10** ± 0.01
CT	NS	9.20 ± 0.03	9.07 ± 0.07	8.98 ± 0.03	8.85 ± 0.03	8.69 ± 0.03	8.61 ± 0.04	8.53 ± 0.02	8.49 ± 0.03
(4˚C ± 2˚C)	SW	9.47 ± 0.04	9.33 ± 0.04	9.26 ± 0.03	8.98 ± 0.01	8.82 ± 0.01	8.83 ± 0.01	8.79 ± 0.02	8.72 ± 0.01

RT: room temperature; CT: cold temperature; NS: normal saline; SW: sterile water; *$p < 0.05$ vs. 0 day and NS (CT); **$p < 0.05$ vs. 0 day and SW (CT).

Table 3. Intra-day and inter-day precision studies (n = 5).

Validation \ Condition	RT NS	CT SW	RT NS	CT SW	RT NS
Intraday (n = 5)	Accuracy (%)	90.86 ± 1.04	91.12 ± 0.32	90.67 ± 0.97	91.15 ± 0.79
	R.S.D. (%)	0.87	0.35	1.07	0.87
Interday (n = 5)	Accuracy (%)	93.45 ± 0.86	101.32 ± 0.82	98.34 ± 0.87	92.56 ± 0.58
	R.S.D. (%)	0.92	0.81	0.88	0.63

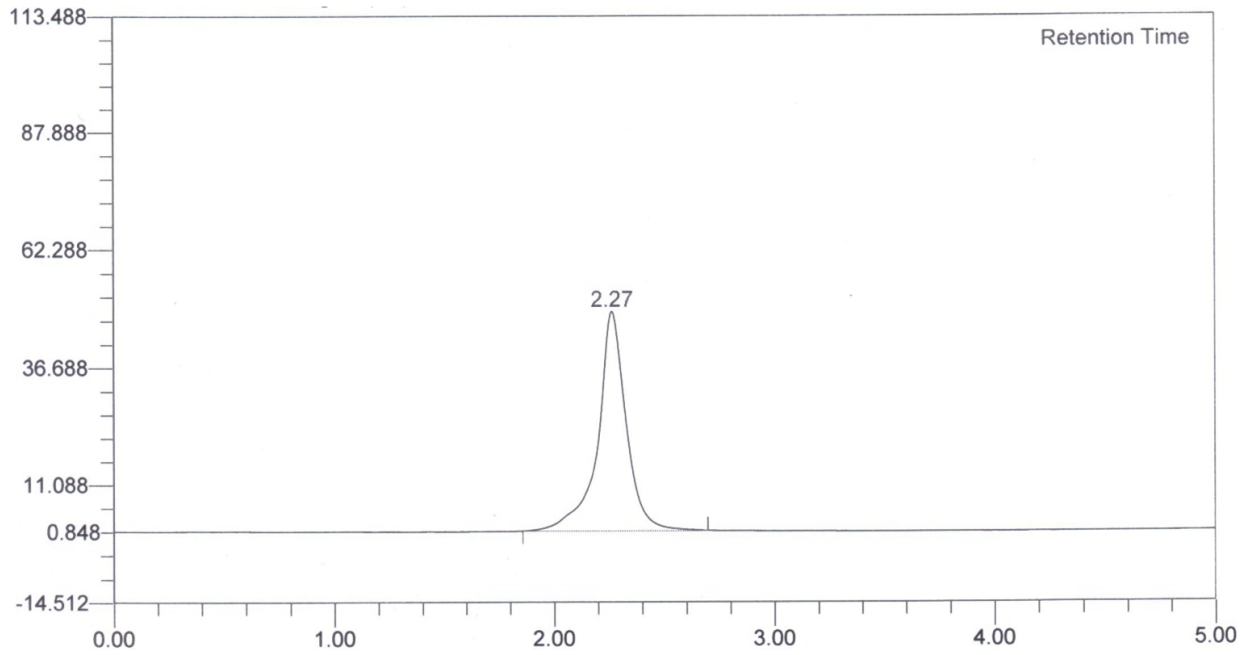

Figure 4. Typical chromatogram of standard solutions (NS and SW) containing ampicillin sodium of 200 µg/mL, Y- axis (Absorbance units), X-axis (Retention time, min).

2.27 min. The initial concentration and the percentage of the remaining concentration which were observed at analytic time of each day during 7 days for each Ampicillin sodium solutions and storage conditions are listed in **Table 4.** The concentration of Ampicillin Sodium slightly changed. 94 % of initial concentration remained in NS and 90 % remained in SW under CT and decreased significantly ($p < 0.05$) to 83 % in NS and to 80 % in SW under RT **(Table 4)**.

4. Discussion and Conclusion

In order to verify the effects of mixing other drugs with antibiotics in a range of intravenous media in hospital pharmacy settings or home-based patient self-administration of peritoneal dialysis solution under home storage conditions, the stability assays are essential for the detection and measurement of interactions, which are often unaccompanied by visual changes [1,2]. The expiry date of IV medications after reconstitution or dilution is often limited to about 24 hrs because of the potential for the breaks in sterility. However, when reconstitution and dilution are carried out in an absolute aseptic environment, according to USP Chapter 797 recommendations [15],

Table 4. The changes of the amount (%) of ampicillin sodium in various solutions (NS and SW) according to each storage temperature (RT and CT) and time (0 - 7 days).

Time (day) \ Condition	RT (25˚C ± 2˚C)		CT (4˚C ± 2˚C)	
	NS	SW	NS	SW
0	100	100	100	100
1	96	95	100	93
2	93	91	96	94
3	90	89	100	93
4	88	86	98	91
5	86	86	100	95
6	84	82	99	94
7	83	80	94	90

the expiry dates of many stable drugs can be extended from 24 hrs to 14 days [15,16]. Extending the expiry date could reduce the wastage of many drugs [17,18] and might increase the convenience for ambulatory patient and home care nurses by eliminating the need for frequent visits to a clinic to obtain additional dose [18] It could especially be convenient for patient who live in regional areas where frequent travel to the local tertiary hospital is difficult [1]. An increasing number of patients are being treated as outpatients, and many of these patients receive their medications or intravenous nutrients by infusion using portable pumps or devices [19]. For example, many patients practice peritoneal dialysis-peritonitis and intraperitoneal administration of antibiotics. This is superior to other intravenous therapies [20] and allows ongoing outpatient treatment, which shortens hospital admission which is beneficial to both patients and to health care system [1]. Therefore, reliable and widely available stability information of antibiotics in the Accufuser® infusion device is clinically important. This study was undertaken to determine the stability of commonly used Ampicillin sodium which was stored in the device according to each recommended storage condition. Visual clarity, pH and concentrations of Ampicillin sodium of the resultant solutions were examined each day during 7 days under different temperature conditions. No visible precipitation or change in color or clarity was observed in both kinds of Ampicillin sodium solutions (in NS and SW) under CT and RT conditions during 7 days. When Ampicillin sodium solutions were prepared under maker's standardized conditions to achieve concentrations of 10 mg/mL in NS and SW, the concentrations of Ampicillin sodium in NS and SW solutions which were stored in an Accufuser® device decreased to 83% and 80% during 7 days in RT conditions, respectively. On the other hand, Ampicillin sodium solutions that were made according to the same conditions and stored in an Accufuser® device decreased to 94% and 90% during 7 days in CT conditions, respectively. Therefore, the Ampicillin sodium solutions (in NS and SW) in an Accufuser® infusion device were both more chemically stable and physically compatible for 7 days at CT rather than RT storage conditions. This result suggests that ready-to-use solutions of Ampicillin sodium in the Accufuser® infusion device can be more safely kept at CT than RT conditions up to 7 days regarding the change of potency and pH of the solutions.

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