Stability of daptomycin 5 mg/mL and heparin sodium 100 units/mL combined in lactated Ringer’s injection and stored in polypropylene syringes at 4 and –20 °C

RAQUEL ORTEGA, ANTONIO SALMERÓN-GARCÍA, JOSÉ CABEZA, LUIS F. CAPITÁN-VALLVEY, AND NATALIA NAVAS

Aim. The stability of an admixture containing reconstituted daptomycin and heparin in lactated Ringer’s injection was evaluated.

Methods. Two samples of the admixture of daptomycin 5 mg/mL and heparin sodium 100 USP units/mL diluted in lactated Ringer’s injection were prepared and divided into 5-mL portions for storage in syringes at 4 and –20 °C for 14 days. The percentage of the initial concentration of the drugs remaining in the syringes was assessed using a high-performance liquid chromatographic (HPLC) method with diode-array detection previously validated as stability indicating for both drugs. Forced degradation studies were performed independently with each drug diluted in lactated Ringer’s injection. One sample from each stored syringe was analyzed in triplicate on days 0, 1, 2, 3, 4, 7, and 14; quality-control samples of each concentration tested were used throughout the analysis. The admixture samples were visually inspected for color, clarity, and the formation of particulate matter.

Results. The HPLC analysis indicated no significant reduction (loss of ≤5%) in the concentration of daptomycin and heparin diluted in lactated Ringer’s injection stored in syringes refrigerated at 4 °C and frozen at –20 °C. None of the chromatographic peaks observed in samples subjected to forced degradation were detected in any sample during the 14-day study. All of the syringe-stored samples remained clear and colorless on visual inspection for the duration of the study.

Conclusion. The admixture of daptomycin 5 mg/mL and heparin sodium 100 USP units/mL was stable when stored in polypropylene syringes for up to 14 days at 4 and –20 °C.
Daptomycin has a unique mechanism of action involving a calcium-dependent dissipation of membrane potential, leading to the release of intracellular ions from the cell and the killing of bacteria. An antibiotic lock solution containing daptomycin needs the presence of calcium, so lactated Ringer injection (containing 3.6 meq/L of Ca ions) is used as the vehicle; this applies when heparin is included as the anticoagulant. The admixture studied here (i.e., daptomycin and heparin prepared in lactated Ringer’s injection) has already been proposed and checked for stability using different concentrations and storage conditions over a short period of time (120 minutes). The use of this admixture as an antibiotic lock solution prompted us to study its stability over a longer duration (up to 14 days). We evaluated the stability of the admixture of daptomycin 5 mg/mL and heparin sodium 100 USP units/mL prepared in lactated Ringer’s injection and stored in polypropylene syringes at 4°C and −20°C for 14 days. The stability of the admixture in both storage conditions was tested using high-performance liquid chromatography (HPLC) with diode-array detection (DAD). This method was specifically developed for this purpose and was validated as stability indicating in accordance with the International Conference on Harmonisation guidelines Q2(R1) and Q1A(R2).

Recent research has proposed analytical methods for the quantification of daptomycin in plasma, either by itself or with different drugs using HPLC with ultraviolet-light or mass spectrometry detection. Heparin is a heterogeneous polysaccharide that has been widely used as an anticoagulant for decades, and there are well-established analytical methods for its routine analysis. However, to the best of our knowledge, no stability-indicating method has been proposed for the simultaneous analysis of the two drugs. The chemical stability of daptomycin with nine medications has been analyzed using HPLC, but the method was not validated as stability indicating. We developed an HPLC method to simultaneously analyze daptomycin and heparin when prepared and stored under typical pharmacy conditions.

**Methods**

**Materials.** Daptomycin, heparin sodium injection, lactated Ringer’s injection, and polypropylene syringes (50 mL and 5 mL) were obtained commercially. HPLC-grade water, acetonitrile, trifluoroacetic acid, and trifluoroacetic acid as a chemical modifier and water in gradient mode; the gradient started from 40% of acetonitrile with 1% trifluoroacetic acid as a chemical modifier and water in gradient mode; the gradient started from 40% acetonitrile with 1% trifluoroacetic acid, it remained constant for 3 minutes, then rose to 45% in 0.1 minute, and then remained constant until 6.9 minutes. This mobile phase was prepared daily and filtered through a 45-µm membrane filter. The temperature of the column compartment was maintained at 40°C. The detection wavelength was set at 210 nm (205–215 nm), using 360 nm (340–380 nm) as the reference wavelength. The retention times were 4.2 minutes for heparin and 7.1 minutes for daptomycin, with a total run time of 8.5 minutes.

Taking into account that the chromatographic analysis was performed at 500 mg/L for daptomycin and 10 USP units/mL for heparin because a 1:10 dilution of the admixture was needed before HPLC analysis (no diluted samples produced saturation of the column or detector), we established a standard linear function by the linear least-squares regression model from 400, 450, 500, 550, 600, 650, and 700 mg/L of daptomycin and from 1, 2, 5, 7, 10, 15, and 20 USP units/L of heparin. These solutions were prepared from dilutions in osmosis-type quality water of appropriate volumes of a solution of 50 mg/mL of reconstituted daptomycin and 1000 USP units/mL of heparin. Four replicates of each concentration were independently prepared and injected into the chromatograph. Linearity was corroborated because the p value of the corresponding lack-of-fit test was greater than 5% (Table 1) according to the guidelines of the Analytical Methods Committee. Table 1 shows the parameters used in the analytical method for the two drugs. The limit of detection (LD) and the limit of quantification (LQ) were estimated from the calibration function; LD and LQ were calculated as 3.3·σ/S and 10·σ/S, respectively, where σ is the standard deviation of the intercept and S is the slope of the calibration function. The precision and the recovery of the method were evaluated by analyzing 10 admixture solutions of daptomycin and heparin at the target concentrations of 5 mg/mL and 100 USP units/mL, respectively. The relative S.D. values were 1.5% for daptomycin and 0.4% for heparin for intraday precision and 1.8% for daptomycin and 1.8% for heparin for interday (five days) precision. The percent recovery ranged from 99.5% to 101.5%. The robustness of the analytical method was evaluated by slightly modifying the mobile phase composition and flow rate, and the changes in the chromatographic variables (retention time, symmetry factor, theoretical...
plate, capacity factor, and concentration tested) were less than 5% for the two drugs analyzed. The system suitability was also corroborated by the high reproducibility value of the injected volume (relative S.D., <0.1%) and the values of the daptomycin and heparin chromatographic peaks (symmetry factors close to 1, theoretical plates greater than 12,000, and resolution greater than 4).

Forced degradation studies were performed independently with each drug diluted in lactated Ringer’s injection. Chromatograms were obtained in order to study the specificity of the method. Degradation was forced using heat (60°C), ultraviolet light (irradiance set at 250 W/m²), acid (adding two drops of 0.1M hydrochloric acid to 1.5 mL of sample), base (adding two drops of 0.1M sodium hydroxide to 1.5 mL of the sample), and oxidation (adding two drops of hydrogen peroxide 1% to 1.5 mL of the sample). All samples were analyzed in duplicate. The peak purity was checked using ChemStation software tools® setting the similarity factor at 995, the recommended value. Although only mild degradation conditions were applied throughout the study, total decomposition of heparin was produced and deduced from the chromatograms, in which no peaks were detected. Subjecting daptomycin samples to the acidic and basic media for 24 hours also resulted in total decomposition of the drug, with no detected peak in the chromatograms. The forced degradation of daptomycin samples with heat or light resulted in several well-resolved unidentified degradation peaks of daptomycin, the largest of which were obtained at 7.7 and 7.8 minutes. The calculated resolution factor (1.8) indicated satisfactory separation between the peaks.

Sample preparation. All of the solutions containing daptomycin were prepared in sterile conditions in a laminar-airflow hood. Commercially available glass vials of daptomycin 500 mg were reconstituted to 50 mg/mL with 10 mL of 0.9% sodium chloride injection. Commercially available heparin sodium 1000 USP units/mL was used in the study. The admixture was prepared in sterile conditions in a laminar-airflow hood. The admixture samples were analyzed in triplicate on days 0, 1, 2, 3, 4, 7, and 14; quality-control samples of each concentration tested were used throughout the analysis. In this study, stability was assumed if the fall in daptomycin or heparin concentration was less than 10% of its initial concentration, obtained immediately after sample preparation (day 0).

Physical assessment. The admixture of daptomycin and heparin in lactated Ringer’s injection was clear and colorless. Samples were visually inspected for particulate matter, clarity, and color changes. This inspection was conducted against a light background and without instruments or magnification.

Results and discussion

All of the admixture samples were stable (Table 2). In addition, the samples remained clear and without color changes throughout the test. The only two chromatographic peaks occurred at 4.2 and 7.1 minutes, corresponding to heparin and daptomycin, respectively. None of the chromatographic peaks observed in samples subjected to forced degradation were detected in any sample during the 14-day study. The DAD system was used to obtain the spectra throughout the chromatographic peaks of heparin and daptomycin, and spectral purity was corroborated for all the samples during the test period.

The study results indicate that the admixture of daptomycin 5 mg/mL and heparin sodium 100 USP units/mL in lactated Ringer’s injection stored in polypropylene syringes is chemically and physically stable for up to 14 days when stored at 4 or −20°C. Such practices are consistent with sterility guidelines for low-risk-level compounded sterile preparations, as outlined in The United States Pharmacopeia.20

Conclusion

The admixture of daptomycin 5 mg/mL and heparin sodium 100 USP units/mL in lactated Ringer’s injection stored in polypropylene syringes is chemically and physically stable for up to 14 days when stored at 4 or −20°C. Such practices are consistent with sterility guidelines for low-risk-level compounded sterile preparations, as outlined in The United States Pharmacopeia.20

### Table 1.

**Factors Characterizing Linearity of Daptomycin and Heparin Assays**

<table>
<thead>
<tr>
<th>Analytical Parameter</th>
<th>Daptomycin</th>
<th>Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (absorbance units)</td>
<td>495</td>
<td>5.7</td>
</tr>
<tr>
<td>Slope (absorbance units/µg/mL)</td>
<td>6.17</td>
<td>23.7</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>99.9%</td>
<td>99.8%</td>
</tr>
<tr>
<td>Probability value for lack-of-fit test</td>
<td>78.5%</td>
<td>56.7%</td>
</tr>
<tr>
<td>Linear dynamic range studied</td>
<td>400–700 mg/L</td>
<td>1–20 units/mL</td>
</tr>
<tr>
<td>Quantification limit</td>
<td>5 mg/L</td>
<td>0.6 unit/mL</td>
</tr>
<tr>
<td>Detection limit</td>
<td>1.5 mg/L</td>
<td>0.2 unit/mL</td>
</tr>
</tbody>
</table>

*Estimated from the calibration function.
units/mL was stable when stored in polypropylene syringes for up to 14 days at 4 and −20 °C.


