"Effect of freezing, long-term storage, and microwave thawing on the stability of cefepime in 5% dextrose infusion polyvinyl chloride bags."

Schlesser, V ; Hecq, JD ; Vanbeckbergen, D ; Jamart, Jacques ; Galanti, Laurence

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Référence bibliographique
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No color change or precipitation in the solution was observed. Cefepime remained stable for 11 days. During this period, the 95% lower confidence limit of the estimated regression line of the concentration-time profile remained above 90% of the initial concentration and the pH value increased slightly without affecting chromatographic parameters.

Within those limits, cefepime may be prepared in advance by a centralized intravenous admixture service; it may be frozen and then thawed in a microwave before use in clinical units.

Introduction

Cefepime, a fourth-generation cephalosporin, is often used in antibiotic infusions for hospitalized patients with critical infections; it is routinely administered in small-volume bags containing 5% dextrose injection or 0.9% sodium chloride injection. Advance centralized preparation of batches of intravenous solutions can improve security, time management, and the speed of drug delivery to the hospital floor for administration.1,2 Freezing the solutions can extend the long-term stability of ready-to-use injectable drugs.3 A disadvantage of such storage is thawing time. Different authors4-5 have used a microwave oven to reduce the time required for thawing a frozen solution, but the effect of microwaving on drug stability is questionable. Although studies6-9 of cefepime stability in sodium chloride and glucose solutions proved that this antibiotic remains stable for at least 24 hours at ambient temperature and for 7 days at 4°C, data about the long-term stability of cefepime after freezing and subsequent microwave thawing are unavailable. The purpose of this study was to investigate the effect of storage and thawing on the stability of cefepime in 5% dextrose injection polyvinyl chloride (PVC) bags.

Materials and Methods

Preparation of Solution

The commercially available powder of cefepime (Lot 97K28-A, 725 mg of L-arginine per gram of cefepime HCl, [Maxipime, Bristol-Myers Squibb, Belgium]) was reconstituted with sterile water for injection, as recommended by the manufacturer, in a laminar-airflow hood; 100-mL Viaflex bags containing 5% dextrose injection (Baxter, Lesines, Belgium) were used to produce solutions containing approximately 2 g of cefepime per 100 mL of solution.

The same commercially available powder of cefepime was used for the extemporaneous preparation of standard solutions.

Chromatographic Apparatus and Conditions

A high-performance liquid chromatographic separation module (Waters Alliance 2690 HPLC Separation Module, Waters Corporation, Milford, Massachusetts) equipped with a multiple wavelength detector (Photodiode Array Detector 996, Waters Corporation) and a data acquisition and processing module (Millennium 210 Chromatograph Manager, Waters Corporation) was used. A µBondapack C18 column (30 cm x 4 mm, 10 µm, Waters Corporation) was the stationary phase.

Table 1. Within-Day and Between-Day Relative Standard Deviation of Tested Cefepime Concentrations.

<table>
<thead>
<tr>
<th>Tested Concentration</th>
<th>Between-Day RSD (%) (n = 15)</th>
<th>Within-Day RSD (%) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>4.73</td>
<td>0.73</td>
</tr>
<tr>
<td>50</td>
<td>3.21</td>
<td>0.63</td>
</tr>
<tr>
<td>100</td>
<td>3.02</td>
<td>0.49</td>
</tr>
<tr>
<td>200</td>
<td>1.92</td>
<td>0.82</td>
</tr>
<tr>
<td>250</td>
<td>0.92</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* Day-to-day RSD on the slope of the standard curve, 0.64%.

RSD = Relative standard deviation.
The mobile phase contained 10% (v/v) acetonitrile (REF 9017, Baker, Deventer, Holland) in 0.0165 M pentane sulfonate (REF P-0299, Sigma, Steinheim, Germany) buffer in water (pH 4). All solvents were prepared from HPLC-grade solvents and purified water (Milli-Q; Millipore, Brussels, Belgium). The flow rate was 1.5 mL/min, and the column temperature was 30°C. The detector wavelength was set at 254 nm.

**Determination of pH**

The pH of the solutions was measured with a pH meter (Radiometer pHM82, Copenhagen, Denmark).

**Validation of the High-Performance Liquid Chromatographic Method**

**Precision:** Assays of control solutions of cefepime (200 mg/100 mL) were undertaken to calculate within-day and between-day variations.

**Linearity of analytical response:** Linearity was evaluated by serial dilutions of cefepime solutions with sterile water for injection.

**Stability indication:** Partially decomposed solutions of the drug were used to assess the stability-indicating capability of the chromatographic method. The cefepime solution (2 g/100 mL) was degraded by heating it at 100°C for 15, 30, and 60 minutes under acidic conditions (HCl, pH 3), initial pH conditions (L-arginine buffer, pH 4.5), and basic conditions (pH 11, NaOH).

**Stability Study**

Five bags each of 2 g/100 mL cefepime solution were prepared as described above, agitated, and stored at -20°C. One month later, the bags containing the cefepime solution were thawed with a validated cycle in a microwave and were then stored at 4°C. For the thawing procedure, an output power of 270 W was applied. The bags were thawed for 13 minutes and were then stirred and thawed again for 7 more minutes to obtain a remaining ice volume of between 1 and 8 cm³. Immediately after preparation of the bags, after thawing (day zero), and after 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 23, and 25 days of storage, 2 mL of solution was withdrawn from each bag by means of a 2-mL polypropylene plastic syringe (Terumo, Haasrode, Belgium) and was placed in glass containers. The admixtures were visually inspected, and the pH of each was measured. The concentration of antibiotic was determined in triplicate.
High-Performance Liquid Chromatographic Assay

**Standard solutions:** Five extemporaneously prepared standard solutions of cefepime in concentrations of 250 mg/100 mL, 200 mg/100 mL, 100 mg/100 mL, 50 mg/100 mL, and 25 mg/100 mL were used to calibrate the high-performance liquid chromatographic system.

**Injections:** A 10-µL quantity of the assay solution and each standard solution was injected into the chromatograph under the conditions described previously.

**Calculations:** Results were automatically calculated by interpolation of a standard curve performed with Millennium software (Waters Corporation); a least squares fit of response (peak height) versus standard concentration was used in the calculations.

**Statistical analysis:** Data are expressed as the mean ± SD. Drug concentrations and pH were followed as a function of time. The drug solutions were considered stable as long as the 95% lower confidence limit of the estimated regression line of the concentration-time profile remained above 90% of the initial concentration, as recommended by the United States Food and Drug Administration.10

Results and Discussion

**Validation of the High-Performance Liquid Chromatographic Method**

**Linearity of analytical response:** Linear regression analysis of the peak height of the drug concentration yielded a correlation coefficient of r > 0.99 in the range of 1 mg/100 mL to 250 mg/100 mL (slope ± percent relative standard deviation [RSD]: 1.008 ± 0.582; intercept ± percent RSD: 0.83 ± 86).

**Precision:** The within-day and between-day relative RDS values are summarized in Table 1.

**Stability indication:** Degraded samples of cefepime solution were assayed to confirm separation of the parent antibiotic from its degradation products. In all conditions, the decomposition product peaks were resolved from the peak corresponding to the intact drug. After heating at 100°C for 15, 30, and 60 minutes and treatment with 0.1 N HCl or 0.1 N NaOH of cefepime solution, the decomposition products were separated from the cefepime peak and eluted earlier than the cefepime peak (Figures 1A, 1B, and 1C).

**Stability of the Antibiotic Infusions**

No color change or precipitation was observed in the antibiotic admixtures when the frozen bags were thawed in a microwave oven under standardized conditions. Subsequent long-term storage at 4°C did not show changes on the same parameters. The retention time for cefepime was approximately 4.0 minutes under the chromatographic conditions described above.

Statistical analysis demonstrated a significant increase (P < 10^-5) in the pH values of solutions under storage (Figure 2). However, the slight pH increase did not affect chromatographic parameters and remained in an acceptable range for perfusion.
There were no additional peaks from possible decomposition products, nor did retention time change.

The initial concentration of the admixture and the changes that took place over subsequent days under storage are shown in Table 2. Statistical analysis shows a significant decrease in cefepime concentration \((P < 10^{-5})\), and the lower 95% confidence limit of the estimated common regression line remained above 90% of the initial concentration until day 11.

**Conclusion**

Cefepime in 5% dextrose injection 100-mL PVC infusion bags may be frozen and thawed in a microwave without major changes that affect concentration. Subsequent storage of solution at 4°C is possible for up to 11 days. Within those limits, cefepime may be prepared in advance by a centralized intravenous admixture service, frozen, thawed, and stored under refrigeration for a few days before use on hospital wards.

**References**


**Table 2. Stability of Cefepime in 100 mL 5% Dextrose Injection in 5 Polyvinyl Chloride Bags.***

<table>
<thead>
<tr>
<th>Storage Time at 4°C (days)</th>
<th>Initial Cefepime Nominal Concentration (1g/100 mL)</th>
<th>Initial Drug (%)</th>
<th>Confidence Interval, 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>104.2 ± 3.2</td>
<td>98.1 - 115.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>112.4 ± 4.0</td>
<td>97.5 - 114.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>105.4 ± 4.7</td>
<td>96.9 - 114.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>107.1 ± 3.5</td>
<td>96.2 - 113.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>101.0 ± 2.9</td>
<td>95.6 - 112.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>102.4 ± 3.4</td>
<td>93.6 - 110.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>95.5 ± 3.5</td>
<td>92.3 - 108.8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>102.0 ± 3.1</td>
<td>90.9 - 107.4</td>
<td></td>
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<tr>
<td>14</td>
<td>98.3 ± 3.6</td>
<td>88.7 - 105.3</td>
<td></td>
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<tr>
<td>16</td>
<td>89.0 ± 4.5</td>
<td>87.3 - 104.0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>93.9 ± 3.0</td>
<td>85.8 - 102.6</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>92.8 ± 3.2</td>
<td>83.5 - 100.7</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>89.7 ± 3.6</td>
<td>81.9 - 99.5</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>93.6 ± 2.6</td>
<td>80.3 - 98.3</td>
<td></td>
</tr>
</tbody>
</table>

*The solution in the bags had been frozen for 1 month at -20°C and was then thawed in a microwave oven with a validated cycle and was placed in final storage for 25 days at 4°C.*