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What is This?
Stability of unused reconstituted bortezomib in original manufacturer vials

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Abstract

Background. Bortezomib is a modified dipeptidyl boronic acid analogue used to treat multiple myeloma, mantle cell lymphoma, and, more recently, renal transplantation graft rejection. As per manufacturer recommendations, bortezomib is to be administered within 8 h of preparation or may be stored for up to 8 h in the vial or a syringe following reconstitution. Preserving unused reconstituted bortezomib beyond these 8 h may allow for cost savings. This study aims to examine the stability of unused reconstituted bortezomib when stored at 4°C for up to 15 days.

Methods. Using an LC-MS/MS assay, the concentration of reconstituted bortezomib was measured at predetermined time points following storage at 4°C in the manufacturer vial. Percent bortezomib remaining at a time point was calculated versus initial bortezomib concentration.

Results. The concentrations of bortezomib were found to be 51.93 ng/mL ± 4.60 after 1 day of storage, 57.40 ng/mL ± 4.77 after 8 days of storage, and 49.43 ng/mL ± 2.85 after 15 days of storage. The percent of bortezomib remaining was 110.53% and 95.19% after 8 days and 15 days, respectively.

Conclusion. Unused reconstituted bortezomib is stable for up to 15 days stored at 4°C in the original manufacturer vial. Such use of bortezomib may improve cost efficiency by reducing bortezomib waste.

Keywords
Bortezomib, stability, multiple myeloma, mantle cell carcinoma, renal transplantation

Introduction

Bortezomib (Velcade®, Millennium Pharmaceuticals) is a modified dipeptidyl boronic acid analog that reversibly inhibits 26S proteosome activity to disrupt cellular protein degradation and multiple signaling cascades.

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ultimately leading to cell death. Bortezomib is currently approved for the treatment of multiple myeloma and mantle cell lymphoma.\(^1\) Additionally, recent research has identified the potential utility of bortezomib in the prevention of graft rejection in renal transplantation.\(^2,3\)

The manufacturer indicates that reconstituted bortezomib should be administered within 8 h of preparation and may be stored for up to 8 h following reconstitution in either the reconstituted vial or a syringe at 25°C.\(^4\) Reconstituted bortezomib is stable in either syringes or single use vials for 5 days when stored at 5°C as a reconstituted solution.\(^5\) However, no data is available on the stability of unused bortezomib remaining after the compounding of doses, when stored in original manufacturer vials. Given the cost of bortezomib ($454 per 1 mg), and the increasing number of indications for its use, there is substantial opportunity for cost savings if unused drug can be stored and used later. The aim of this study was to investigate the stability of reconstituted bortezomib in the manufacturer vial stored at 4°C for up to 15 days following reconstitution.

**Methods**

**Sample preparation**

A 3.5 mg vial of bortezomib\(^a\) was reconstituted with 3.5 mL of normal (0.9%) saline\(^b\) under routine conditions in the cancer center pharmacy to yield a clear and colorless solution at a bortezomib concentration of 1 mg/mL. A dose of 2.5 mg of bortezomib was removed for patient administration and the remaining 1 mL was delivered to the laboratory for analysis. After a baseline analysis sample was prepared, the vial was stored at 4°C for duration of study. The baseline sample is necessary as it is the comparator for the stored samples. An aliquot of standard stock 1 mg/mL bortezomib in 55% acetonitrile\(^c/45\%\) water\(^d/0.1\%\) formic acid\(^e\) was thawed at room temperature. Bortezomib in solvent is stable through three freeze/thaw cycles when stored at −70°C and is discarded after the third thaw.\(^1\) Both the drug sample and standard stock samples were diluted 1:10,000 in triplicate microfuge tubes containing 55% acetonitrile/45% water/0.1% formic acid (two serial 1:100 or 10 μL bortezomib into 990 μL solvent dilutions were performed). These tubes were vortexed after each dilution. Finally, 1.0 mL of water was added to each tube and vortexed to improve peak shape during LC-MS/MS analysis by more accurately simulating the chromatographic solvent conditions. The final concentration was 50 ng/mL.

**LC-MS/MS assay**

The HPLC\(^f\) consisted of a model 1200 binary pump, vacuum degasser, thermostatted column compartment held at 35.0°C, and a model 1100 thermostatted autosampler held at 35.0°C. The HPLC was coupled directly to a model API 4000 triple quadrupole mass spectrometer equipped with a Turbo V\(^\text{TM}\) atmospheric pressure ionization source fitted with the electrospray probe\(^h\). A 30 × 3.0 mm\(^2\) Ultra II Biphenyl (Restek) 2.2 micron HPLC column was the analytical column. The mobile phase solvents were: (A) Millipore Type I water\(^b\) and (B) HPLC grade acetonitrile\(^c\). The solvents were mixed 70% A/30% B and delivered isocratically at 600 μL/min. Run time was 2.5 min. Mass spectrometry data were obtained in negative ion mode. The multiple reaction monitoring (mrm) transitions were m/z 383.2 → m/z 322.1 for bortezomib. Negative ion instrument parameters were: curtain gas (CUR) 14 units, gas1 (GS1) 38 units, gas2, collision gas (CAD) 6 units, interface heater (IHE) 475°C, nebulizer current −1.0, declustering potential (DP) −57 V, entrance potential (EP) −8 V, collision exit potential (CXP) −9 V, collision energy (CE) −25 V, dwell 220 ms. Quantitation of triplicate samples was determined by one-point calibration where the mean concentration of the drug sample was compared to that of the reference standard. Concentrations within 15% of the day 1 concentration were considered stable.

**Results**

Table 1 provides stability data of bortezomib (1 mg/mL) stored at 4°C over 15 days, tested at a diluted concentration of 50 ng/mL. The bortezomib samples at days 8 and 15 retained more than 95% of the baseline (24 h after reconstitution) concentration. The variability identified in the results on days 8 and 15 are well within the accepted degree of assay variability.

**Discussion**

Bortezomib reconstituted to the manufacturer’s recommendation was found to be stable up to 15 days when

<table>
<thead>
<tr>
<th>Duration of storage at 4°C</th>
<th>Concentration of bortezomib (mean ± SD, ng/mL)</th>
<th>Percent of bortezomib remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>51.93 ± 4.60</td>
<td>N/A</td>
</tr>
<tr>
<td>8 days</td>
<td>57.40 ± 4.77</td>
<td>110.53</td>
</tr>
<tr>
<td>15 days</td>
<td>49.43 ± 2.85</td>
<td>95.19</td>
</tr>
</tbody>
</table>
refrigerated at 4°C. This finding allows for reconstituted bortezomib vials with remaining drug to be preserved and used at a later date, generating cost savings. This economic impact is substantial, given the cost of bortezomib at an average wholesale price of $1589 per 3.5 mg vial. An estimated cost-savings opportunity, based on conservative assumptions of bortezomib waste was calculated. Assuming that a reconstituted vial with 1 mg of remaining drug is likely to be discarded once every third day in any given 5-day treatment week, over the course of 1 year, a total of 87 mg of bortezomib would likely be discarded, for a loss of $39,346. Based on these findings, the ability to reuse these remaining aliquots of bortezomib has the potential to reduce economic losses due to waste.

While HPLC assays require degradation controls as part of assay validation, to assess for degradation peaks co-eluting with parent compound,\textsuperscript{5,6} the coupling of mass spectrometry to liquid chromatography provides excellent specificity, by accurately identifying the mass of the analyzed compound. Therefore, degradation controls were not performed.

**Conclusion**

Unused reconstituted bortezomib is stable for up to 15 days stored at 4°C in the original manufacturer vial.

**Funding and Acknowledgments**

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**Notes**

\textsuperscript{a}Velcade 3.5 mg, Millennium Pharmaceuticals, Cambridge, MA, lot # 100783.  
\textsuperscript{b}0.9% sodium chloride injection 100 mL, Baxter, Deerfield, IL.  
\textsuperscript{c}Fisher Scientific A998-4 HPLC grade, Fisher Scientific, Hampton, NH.  
\textsuperscript{d}MilliQ UV Plus, Millipore Corporation, Billerica, MA.  
\textsuperscript{e}Acros Organics 99+ %, ThermoFisher Scientific, Waltham, MA.  
\textsuperscript{f}Agilent Technologies, Palo Alto, CA.  
\textsuperscript{g}Applied Biosystems/MDS Sciex, Concord, Ontario, Canada.  
\textsuperscript{h}Millipore Corporation, Billerica, MA.

**References**