Stability study of Bortezomib (Velcade) with limit test for all degradation products

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Background

Bortezomib (Velcade\(^9\)) costs approx. 1000 € per vial and is available as a lyophilized powder, which must be reconstituted before administration. The resulting solution is stable for 8 hours according to the SPC, and leftovers therefore cannot be used on subsequent days. This imposes a significant economic loss on hospital budgets. Several studies have shown that the reconstituted drug is stable for > 24 hrs, but none of these have contained identification and quantification of the degradation products formed during storage.

Materials and methods:
The analytical method was based on the work by Srinivasulu and colleagues (1). The storage conditions were 5 °C ± 3 °C, protected from light, and the study consisted of the following measurements: Assay, DPs and visual inspection. Measurements were conducted at 0, 1, 3, 7, 10 and 14 days with analysis of the same three vials of Bortezomib per timepoint. The acceptance criteria for the study were: Assay: 95.0 - 105.0 % of initial value, Bortezomib impurity E: < 3.0 %, other impurities: < 0.5 %, summarized other impurities: < 2.0 % and a clear and particle free liquid.

**Objective**

To conduct a stability study of reconstituted Velcade 2.5 mg/mL in the manufacturer’s vial, with identification and quantification of all degradation products.

**Results**

**Stability study**

A. Visual inspection:
- No change throughout study

B. Degradation products (figure 2)
- No increase in amount of known impurities
- Small increase in one unknown impurity, concentration below specification limit at t=14 days.

C. Assay (figure 3)
- Large deviations due to sampling error caused by viscosity and low sample volume.
- 95% confidence interval of regression line ≥ 105.0 % after 13 days.

**Conclusion**

Bortezomib (Velcade) 2.5 mg/mL is stable for at least 12 days for 5°C when stored in the manufacturer’s vial.

References:


No conflict of interests

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**Identification of degradation products**

The degradation pathway of Bortezomib (figure 1) was confirmed by stress tests and the identity of the degradation products was confirmed by comparison with literature values and UPLC-MS analysis. Furthermore, the identity of Impurity E was confirmed by comparison with the synthesized compound.

**Table 1:** Comparison of retention times from literature (1), our study HPLC/UV method and confirmatory UPLC-UV-MS analysis. The theoretical and observed masses are shown.

<table>
<thead>
<tr>
<th>Imp ID</th>
<th>Retention (time min)</th>
<th>masses (Da)</th>
<th>Literature HPLC-UV</th>
<th>Study HPLC-UV</th>
<th>UPLC-UV-MS</th>
<th>Theoretical</th>
<th>Observed</th>
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<td>356,4</td>
<td>379,2</td>
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<tr>
<td>D</td>
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<td>356,4</td>
<td>379,2</td>
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</table>

\(^1\)Conducted on a Waters Aquity UPLC system with a QDA detector. Closum: Aquity UPLC BEH 1.8μm, 2.1 x 100mm, mobile phase A: H2O w 0.1 % Formic acid, mobile phase B: acetonitrile with 0.1 % Formic acid. Flow: 0.6 mL/min, gradient: 0 min; 90 % A, 8 min; 25 % A, column temperature 35°C, MS cone voltage 15 V, probe temperature 600°C, capilary positive 0,8 kV, mass range 80-450 Da.

\(^2\)Retention times (min) = observed retention time - the retention time of Bortezomib.

**Figure 1:** The degradation pathway of Bortezomib in solution.

**Figure 2:** Chromatograms of the Bortezomib vial, analyzed at t=0, t=3, t=7, t=10 and t=14 days.

**Figure 3:** Bortezomib assay results. No significant difference between the slope (p=0.82) or the intercept (p=0.47) of the individual data series was found, and therefore the data was pooled. The resulting regression line is shown in brown, along with the 95% confidence band of the line (dotted, brown lines).

The individual data points are shown as mean ± S.D. (3 replicates), and the black, dotted lines show the specification limits (95.0 ± 105.0 %). The statistical analysis was performed using GraphPad Prism software (v. 7.0).