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

K. B. Nissen[1], L. B. Jørgensen[1], M. N. Petersen[1], G. Andersen[1]

[1]Odense University Hospital, Hospital Pharmacy Fyn, Odense, Denmark. Email: klaus.nissen@rsyd.dk

Background

Bortezomib (Velcade®) 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

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 litterature values and UPLC-MS analysis. Furthermore, the identity of Impurity E was confirmed by comparison with the synthesized compound. (table 1)

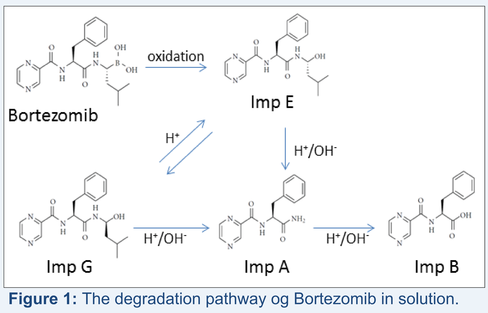

Figure 1: The degradation pathway og Bortezomib in solution.

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.

Imp ID	Retention times (min)			Masses (Da)	
	Litera-ture HPLC-UV[a]	Study HPLC-UV[a]	UPLC-UV-MS[b]	Theo-retical	Observed
A	6,0	6,0	2,8	270,3	293,2 $[M + Na]^+$
B	11,7	11,7	3,4	271,3	272,14 $[M+H]^+$
E	22,5	22,8	4,4	356,4	379,2 $[M + Na]^+$
G	25,8	27,7	4,7	356,4	379,2 $[M + Na]^+$
E synthetic	22,5	22,8	4,4	356,4	379,2 $[M + Na]^+$

[a]As described in (1)

[b]Conducted on a Waters Aqcuity UPLC system with a QDA detector. Coloum: Aqcuity UPLC BEH 1.8µm, 2.1 x 100mm, mobile phase A: H_2O 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 temperatrure 35°C. MS cone voltage 15 V, probe temperature 600°C, capilary positive 0,8kV, mass range 80-450 Da.

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.

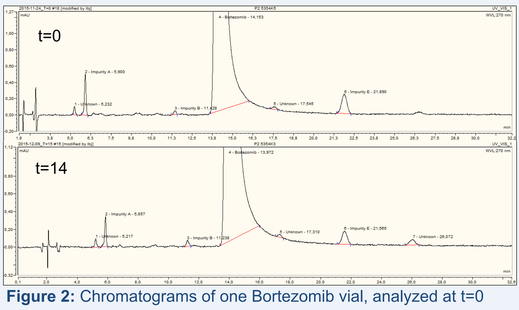

Figure 2: Chromatograms of one Bortezomib vial, analyzed at t=0 days and t=14 days .

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.

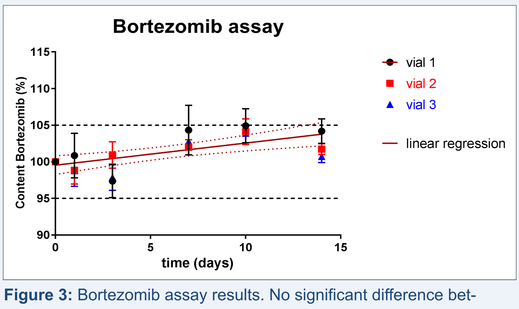

Figure 3: Bortezomib assay results. No significant difference between the slope (p=0,62) or the y-intercept (p=0.47) of the individual data series was found, and therefore the data was pooled. The resulting regression line is shown in brown, along with the 95 % confidence band of the line (dotted, brown 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)

Conclusion

Bortezomib (Velcade) 2,5 mg/mL is stable for at least 12 days for 5°C when stored in the manufacturer's vial.

No conflict of interests

References:

(1). Srinivasulu K, Naidu MN, Rajasekhar K, Veerender M, Suryanarayana MK. Development and Validation of a Stability Indicating LC Method for the Assay and Related Substances Determination of a Proteasome Inhibitor Bortezomib. Chromatography Research International. 2012;2012:Article ID 801720, 13 pages.