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IJPBS |Volume3| Issue 1 |JAN-MAR |2013|449-458

Research Article
Pharmaceutical Sciences

CHEMICAL STABILITY OF BORTEZOMIB SOLUTIONS IN ORIGINAL MANUFACTURER VIAL AT ROOM TEMPERATURE AND IN SYRINGE AT 4^{0}C

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ABSTRACT

Bortezomib is a drug used in the treatment of myeloma multiple. The experiments carried out demonstrate that bortezomib is cytotoxic for different types of neoplastic cells and reduces the tumor-like growth "in vivo" in many preclinical models of tumor, including myeloma multiple. In a recent study, was evaluated the effectiveness and security of the subcutaneous administration of bortezomib as opposed to the conventional intravenous administration. It is necessary to emphasize that the concentration of the solution used for subcutaneous administration is 2.5 mg mL^{-1}, unlike the dissolution for intravenous injection is prepared to 1 mg mL^{-1}. The objective of this work is to evaluate the stability of the bortezomib dissolution reconstituted with NaCl 0.9 % at 2.5 mg mL^{-1} concentration in original manufacturer vial at room temperature and in syringe at 4^{0}C.

KEYWORDS

Bortezomib, stability study, room temperature, syringe, HPLC, subcutaneous administration.

INTRODUCTION

Bortezomib is a medicine that uses in the treatment of the multiple myeloma. His mechanism of action defines like inhibitor of the proteosome. According to the note technical of the product, has designed specifically to inhibit the activity of the 26S proteosome in cells of mammal. The 26S proteosome is a protein complex of big size that degrades certain proteins. The inhibition of the 26S proteosome affects to multiple waterfalls of intracellular signaling, what originates in the last resort the death of the neoplastic cell. The experiments realized show that the bortezomib is cytotoxic for distinct types of neoplastic cells and that reduces the growth of tumor "in vivo" in many models preclinical of tumor, included the multiple myeloma.

The multiple myeloma constitutes, by frequency, the second hematological tumor after the lymphoma.

The first clinical assays with bortezomib effected in patients with refractory multiple myeloma or in relapse [1, 2]. In them the tax of answer was of 35% (10% of complete answer) using the bortezomib in dose of 1,3 mg m^{-2} by intravenous via the days 1, 4, 8 and 11 each 21 days, being able to associate with dexamethasone in case of incomplete answer. These data were confirmed in an essay in phase III [3] with 669 patients with refractory multiple myeloma, in those who the bortezomib was more effective that the conventional treatment with dexamethasone in high doses, so much regarding the tax of answer (a 43 in front of 18%) like the time until the progression (6, 2 in front of 3, 4 months) and the

global survival to the year (a 80 in front of 67%). Later they have carried out other clinical essays in combination with other cytotoxic drugs, as well as in different profiles of patients with multiple myeloma.

At present, the indications approved according to technical note by the European Medicine Agency are:

- in combination with melphalan and prednisone in the treatment of patients with multiple myeloma that have not been previously treaties and that are not candidates to receive treatment with high dose of previous chemotherapy to a transplantation of bone marrow.
- as monotherapy in the treatment of the multiple myeloma in progression in patients that have received previously at least a treatment and that have been subjected or are not candidates to a transplantation of bone marrow.

In a recent study carried out by Moreau et al. [4], was evaluated the efficiency and security of the administration subcutaneous of bortezomib. It treats of a clinical essay phase III of no inferiority in front of the conventional intravenous administration, both to a dose of 1, 3 mg m^{-2} the days 1, 4, 8 and 11 each 21 days in patients with multiple myeloma in relapse. The study was realized with 222 patient to receive subcutaneous bortezomib (n=148) or intravenous (n=74). The tax of global answer after four cycles was of 42% in both groups, showing the no inferiority. Neither had they found significant differences in the time until progression neither in the global survival to a year between both groups. The incidence of hematological adverse effects was similar in both groups. However it observed a decrease in the incidence of neuropathy peripheral in the patients to which administered by subcutaneous. The results of this study show that the subcutaneous administration is a valid alternative to the intravenous, as it presents the same profile of efficiency and better profile of security. It is appropriate to underline that the concentration of the solution for subcutaneous administration is 2.5 mg mL^{-1}, unlike the intravenous that prepares to 1 mg mL^{-1}, without that this affect of significant manner to the pharmacokinetic parameters of the bortezomib. The subcutaneous administration offers some advantages on the intravenous, like the absence of need of catheter intravenous and the possibility of domiciliary administration, which can signify in important reductions of sanitary costs as well as improvements in the comfort of the patients.

Each vial of velcade(R) contains 3.5 mg of bortezomib in dust for solution injectable. The recommendations for the reconstitution that appear in the technical note are the destined to the administration by direct intravenous route to a final concentration of 1 mg mL^{-1}. According to this, the chemical and physical stability of the reconstituted solution is of 8 hours to 25^0C conserved in the vial original and/or a syringe before the administration. The information about the real stability of the drugs after the reconstitution or dilution in different vehicles is crucial for the preparation units. This is even more important in the case of the antineoplastic drugs, since in his majority prepare to individual dose by patient, and treats of drugs with high toxicity and narrow therapeutic margin. Unfortunately, these data no always are available since the laboratories manufacturers frequently limit his results of stability to a maximum of 8, 12 or 24 hours, by reasons of microbiological stability or because the studies carry out only in these short periods of time, the only demanded to obtain the register by part of the regulatory agencies.

In the majority of the countries, the antineoplastic drugs prepare in units centralized inside the pharmacy hospital, under strict conditions of asepsis, by what, elimination the microbial pollution, the important stability is the

physical-chemistry that has to take into account the distinct concentrations, vehicles, primary conditionings or other environmental conditions that can affect to this stability in the practical usual clinic. In this sense, has published recently a guide [5] for the design of practical studies of stability of antineoplastic drugs by means of European consensus.

At present we have of diverse studies of stability of the solution of bortezomib 1mg/ml for intravenous administration [6-10].

In a previous work, we investigated the stability of bortezomib solution (2.5 mg mL^{-1}) in the manufacturer vial stored at 4^0C in the dark for up to 30 days following reconstitution [11].

The aim of this investigation was to assess the stability of bortezomib solution (2.5 mg mL^{-1}) in the manufacturer vial stored at room temperature and in syringe at 4^0C, both in the dark.

EXPERIMENTAL

INSTRUMENTATION

HPLC analysis was performed at room temperature (~25^0C) using an Agilent Technologies, model LC 1220 Infinity including isocratic pump (maximum pressure 600 bar), manual injector valve 20µL and detector with variable wavelength (working wavelength at 270 nm). The signal from the detector was recorder and integrated with a PC HP Pro 3010 Desktop VN934EA; a Zorbax Eclipse XDB-C18, 4.6x250 mm, (5 µm) column was employed. The mobile phase consisted of acetonitrile: water (40:60, v/v).

MATERIALS

Bortezomib is commercialized by Millennium Pharmaceuticals (Mass, USA) in the US and Janssen-Cilag in Europe under the trade name Velcade. The vials are reconstituted with 1.4 mL of sterile NaCl 0.9% to obtain 2.5 mg mL^{-1} solution of bortezomib and stored in vial at room temperature and in syringe at 4^0C. The product information states that reconstituted bortezomib at 1.0 mg mL^{-1} is stable for 8 hours when stored at <25°C and protected from light, and for 3 hours in a syringe. No information was available at this moment about the stability of bortezomib solution at 2.5 mg mL^{-1} concentration stored in vial at room temperature protected from light and at the same concentration stored in syringe protected from light and stored at 4^0C.

Stability study in vial at room temperature

On study day 0, two vials of bortezomib were each one reconstituted with 1.4 mL of 0.9% NaCl to prepare solutions of concentration 2.5 mg mL^{-1}. One of the vials was stored into vial at room temperature for stability study and the other was stored in the freezer for daily preparation of standards, both protected from light.

Stability study in syringe at 4^0C

On study day 0, two vials of bortezomib were each one reconstituted with 1.4 mL of 0.9% NaCl to prepare solutions of concentration 2.5 mg mL^{-1}. One of them was stored into syringe in the refrigerator at 4°C for stability study and the other was stored in the freezer for daily preparation of standards; both (vial and syringe) were protected from light.

Physical stability at room temperature

Vial and syringe

On all days studied, samples were drawn for analysis of concentration and were inspected visually for changes in colour and presence of particulate matter (vial and syringe).

Bortezomib analysis

Vial

On each study day (0, 1, 5, 8, 12, 19, 26, 33), a 100 µL aliquot of thawed solution was used to

prepare standards with final concentration of 50, 125 and 250 µg mL^{-1} into NaCl 0.9%. These standards combined with a blank solution allowed the construction of a calibration curve. On the other hand, a quality control sample with bortezomib concentration of 125 µg mL^{-1} was prepared from the solution stored at room temperature in the dark. Triplicate 20 µL quantities of each prepared sample, quality control sample, standards and blank were injected manually in the column. The area under the bortezomib peak at 270 nm was subjected to least squares linear regression, and the bortezomib concentration in each sample was determined by interpolation from the calibration curve. The bortezomib was eluted at 3.3 minutes with a flow rate of 1.5 mL min^{-1}.

Syringe

On each study day (0, 2, 7, 16, 23), analysis of bortezomib stored in syringe at 4^0C in the dark was carried out at the same manner described into before paragraph.

Accelerated degradation analysis

Five different studies were carried out for this purpose over bortezomib solution stored at room temperature: acid, base, heat, hydrogen peroxide and sodium hypochlorite.

RESULTS AND DISCUSSIONS

Bortezomib stability study

Physical stability

All solutions, as reconstituted in the original manufacturer's glass vials and syringe, were initially clear and colourless and remained so for the duration of the study. Also, no visible particles were observed in any solution throughout the study period.

Bortezomib analysis

During the study period, the concentration in all study sample retained at least 92 % of the initial concentration of bortezomib both in original manufacturer's vial and syringe. **Table 1** provides stability data of bortezomib (2.5 mg mL^{-1}) stored at room temperature in the dark over 33 days, tested at a diluted concentration of 125 µg mL^{-1}.**Table 2** shows the same study realized over the sample contained in syringe at **4^0C** in the dark.

Accelerated degradation analysis

The subsequent studies were made over bortezomib solution stored at room temperature.

pH study

The ultraviolet spectrum of bortezomib (200-365 nm) shows no variation in acid, neutral and basic medium with a maximum wavelength at 270 nm in all cases. On the other hand, an aliquot of 300 µL of 125 ppm of sample were added different amounts of HCl 0.1 M (50, 100, 150, 200 and 300 µL); the concentration of bortezomib in these samples were (107.14, 93.75, 83.33, 75.0 and 62.5 ppm), the chromatograms of these samples let to obtain a calibration graph with a slope similar to the chromatograms obtained when were added different amounts of NaOH (between 0.01-0.1 M) for to obtain the same concentration of bortezomib in basic medium. Both slopes were of the same order with respect to slope obtained in neutral medium. The higher difference observed in these chromatograms were the displacement of the retention time as consequence of diverse peaks corresponding to a degradation products, principally in basic medium as can be seen in **Figure 1**.

On the other hand, in **Figure 2** was represented the variation of the signal with respect to time for different additions of HCl and NaOH solutions, measured each one at retention time appears in **Figure 1**.

Heat study

A sample of 125 ppm of bortezomib was heat at different temperatures (40^0, 60^0, 70^0, 100^0C) during different times. No significant change was observed when the sample is heated at 40^0C or 60^0C during different time periods still 180 min.

In the case of 70^{0}C and 100^{0}C the chromatograms of bortezomib obtained after heat the sample during different time periods appear clearly the degradation products at retention time between 1.3-3.9 min. The results obtained were shown in **Figure 3** for 100^{0}C.

Influence of hydrogen peroxide

Degradation of bortezomib with hydrogen peroxide occurs quickly. At ambient temperature, 300 µL of 125 µg mL^{-1} of bortezomib solution was degraded when 20, 50, 100 and 200 µL of hydrogen peroxide solution 0.03% were added and the degradation products appear to 2.2 and 4.9 min. **Figure 4** shows the evolution of the signal for retention time 3.3 and 4.9 with different amounts of H_2O_2 added.

Influence of sodium hypochlorite

Degradation of bortezomib with sodium hypochlorite also occurs quickly (**Figure 5**). At ambient temperature, to 300 µL of 125 µg mL^{-1} of bortezomib solution were added 20, 50 and 100 µL of sodium hypochlorite solution 0.02 M, and the evolution of peak area were studied with the time (**Figure 5a**) at R_t=3.3 min and **Figure 5b**) at R_t=4.9 min). As can be seen from this **Figure** bortezomib was degraded completely when 50 µL of sodium hypochlorite solution 0.02 M was added and degradation product appears at 4.9 min when the sample is chromatographied immediately. After 10 min, this degradation product was newly degraded in other products (Rt= 2, 2.2, 8.5 min).

Table 1. Stability of bortezomib at room temperature in the dark

Study day	[bortezomib] (mean ± SD, µg mL^{-1})	Percent of bortezomib remaining
Day 0	124.5 ± 0.6	99.6
Day 1	124.9 ± 0.6	99.2
Day 5	122.4 ± 0.7	97.9
Day 8	120.7 ± 2.6	96.6
Day 12	121.9 ± 2.1	97.5
Day 19	115.5 ± 2.0	92.4
Day 26	113.8 ± 1.4	92.0
Day 33	121.0 ± 1.0	96.8

Table 2. Stability of bortezomib at 4^{0}C stored in syringe in the dark

Study day	Concentration of bortezomib (mean ± SD, µg mL^{-1})	Percent of bortezomib remaining
Day 0	126.0 ± 2.0	100.8
Day 2	124.4 ± 1.0	99.5
Day 7	127.2 ± 1.0	101.7
Day 16	123.7 ± 2.6	99.0
Day 23	124.6 ± 0.6	99.7

Figure 1. Variation of retention time as function of NaOH solution added at different concentrations.

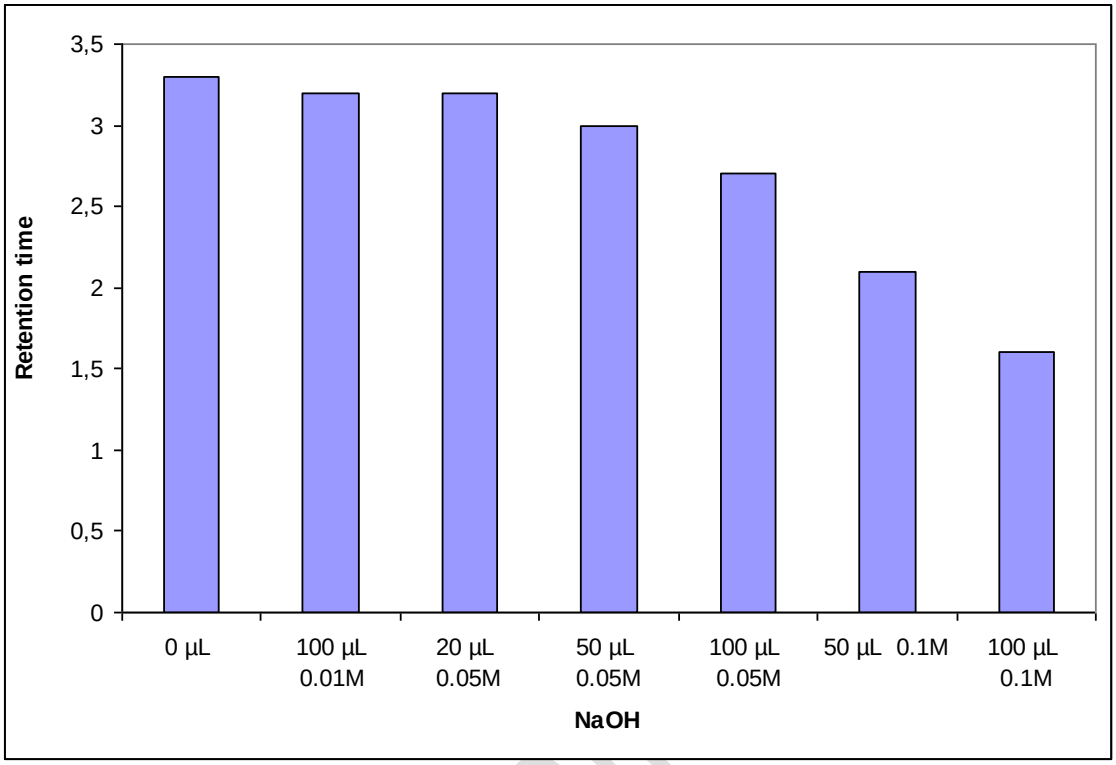
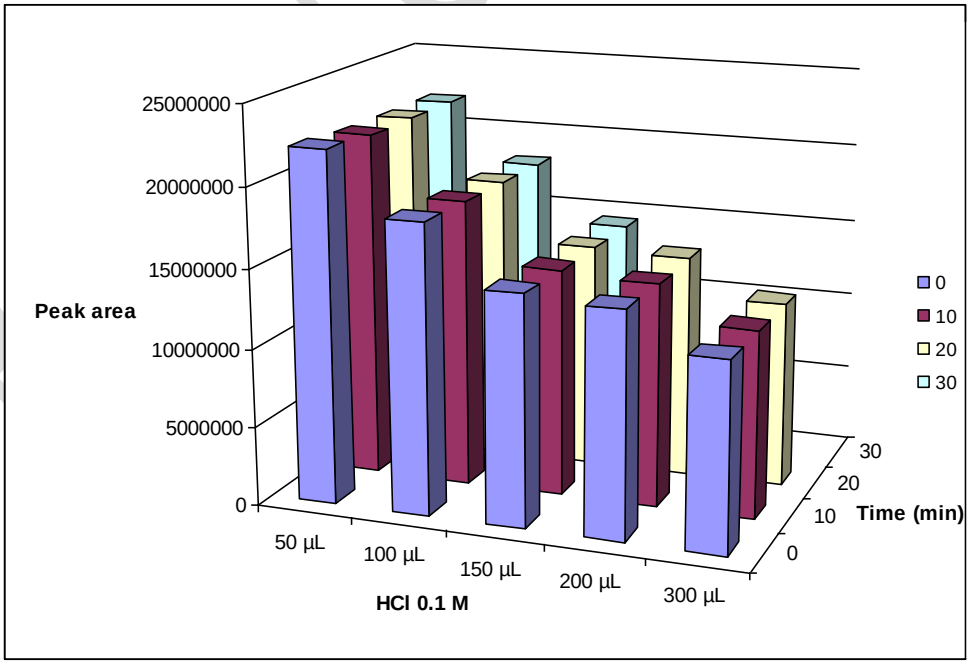


Figure 2. Variation of the bortezomib signal with the time for different additions of HCl 0.1 M (a) and NaOH(b)

a)



b)

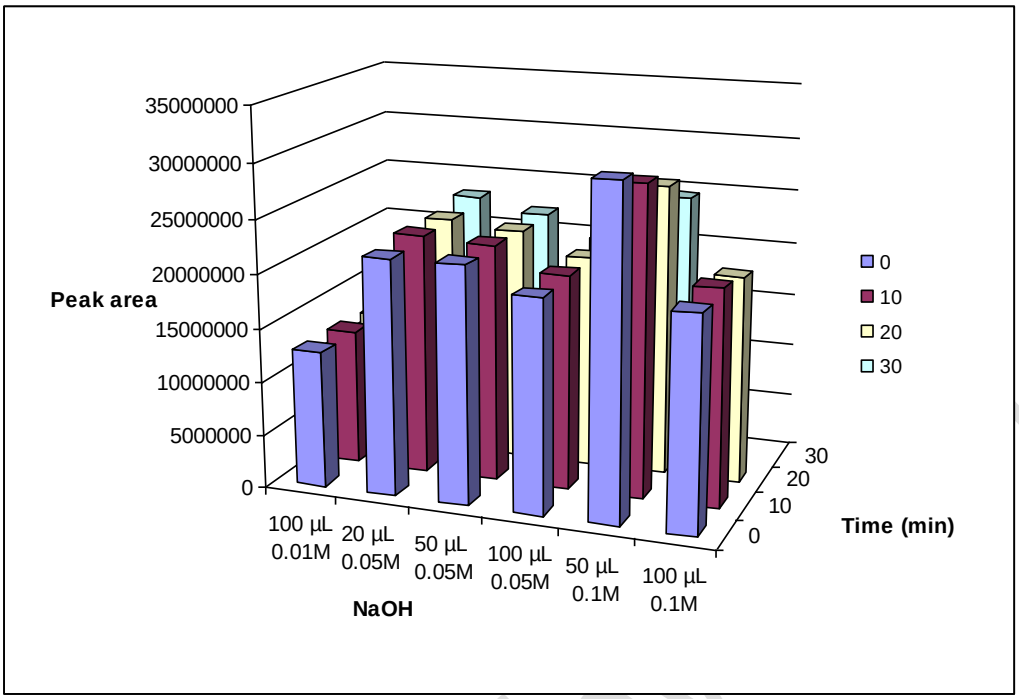


Figure 3. Evolution of different degradation products with respect the heat time at 100^{0}C

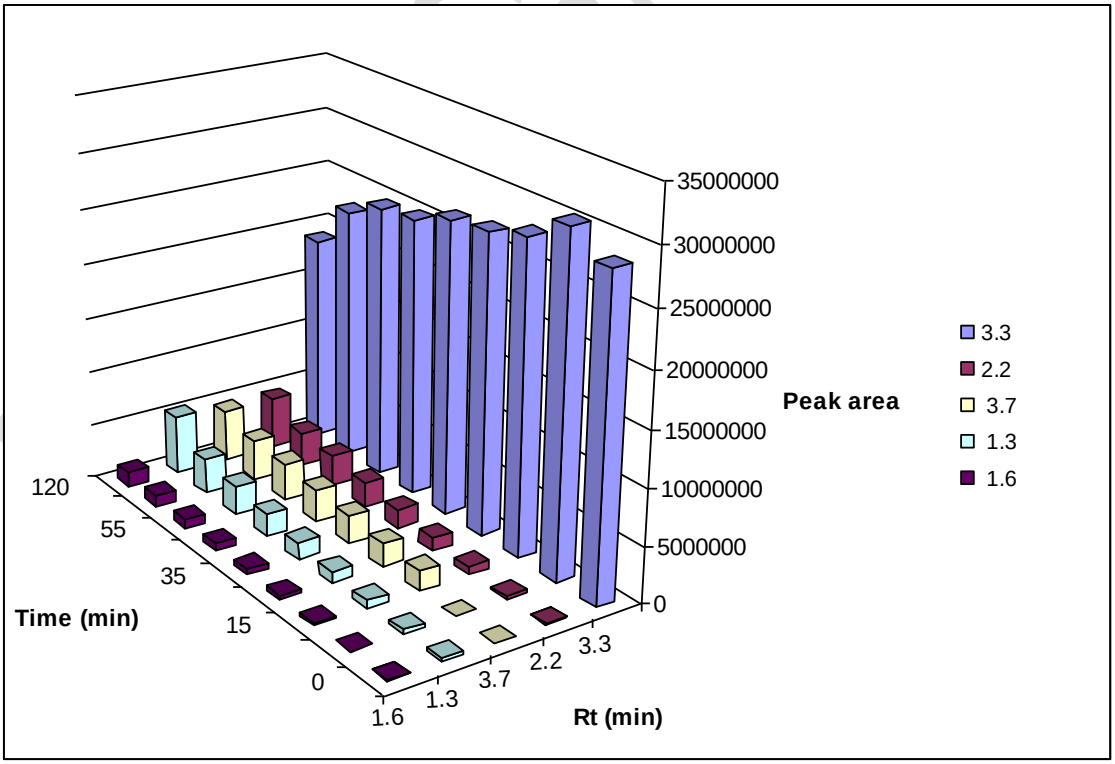


Figure 4. Influence of different amounts of H_2O_2 0.03% solution over peak areas at time 0 min

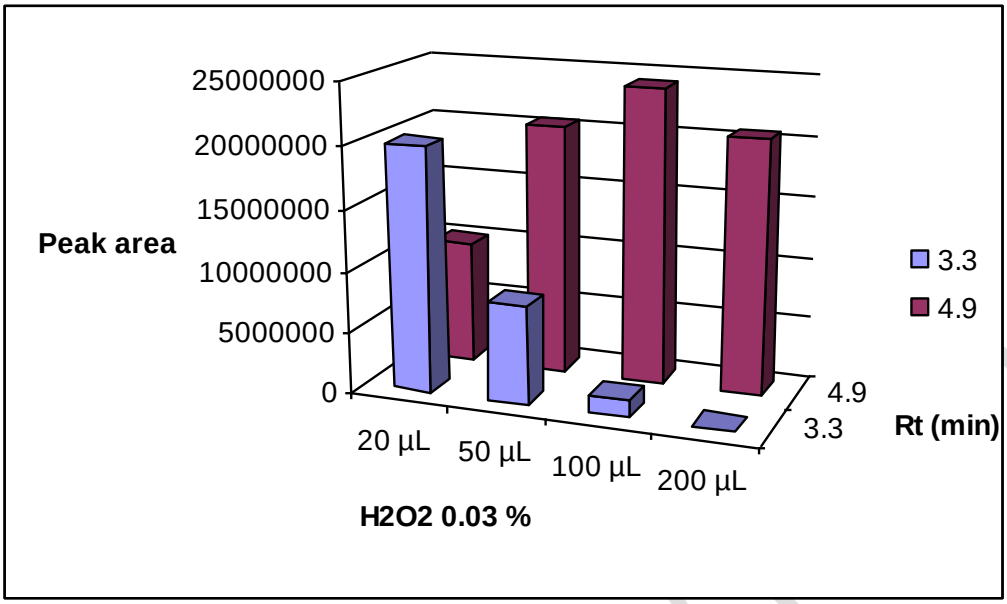
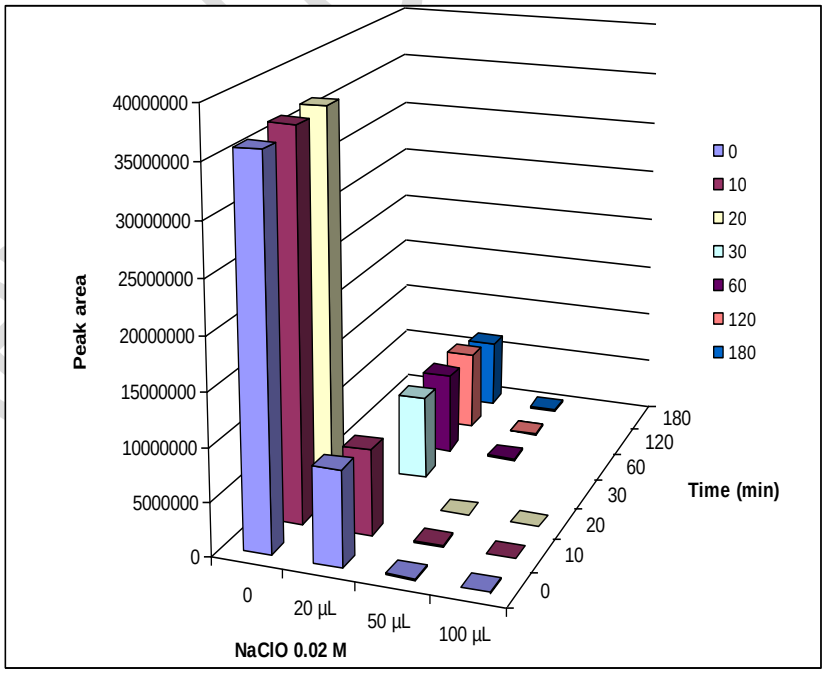
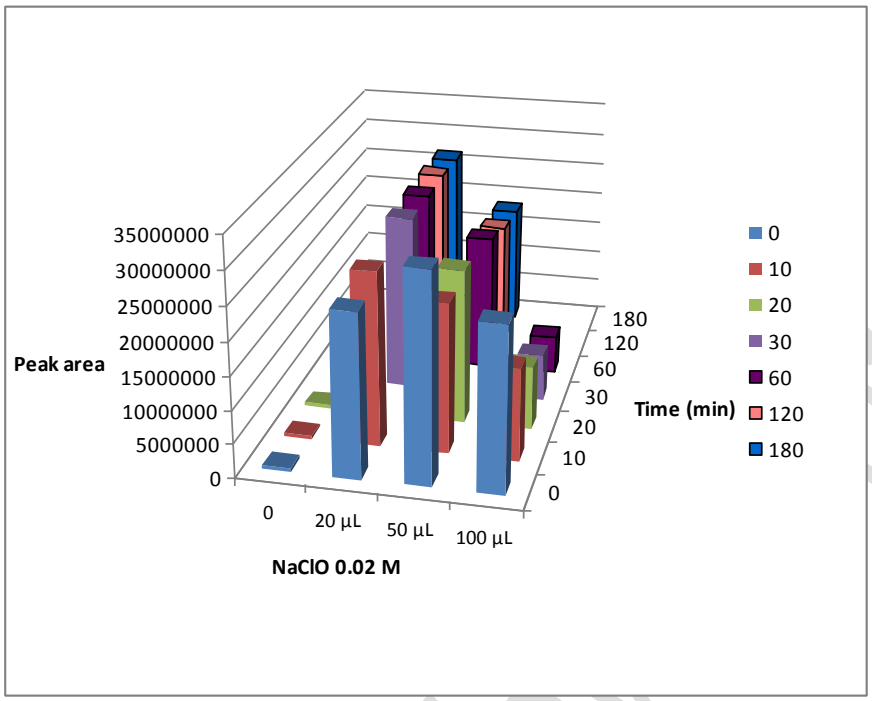


Figure 5. Variation of peak area over bortezomib signal as function of the time when different amounts of NaClO 0.02 M solution were added: a) At R_t=3.3 min; b) At R_t=4.9 min

a)



b)



CONCLUSIONS

Reconstituted bortezomib 2.5 mg mL^{-1} for subcutaneous administration was physically and chemically stable at least for 33 days in the original manufacturer vial at room temperature and at 4°C at least for 23 days in syringe, both in the dark which could make an important contribution towards reducing drug waste and, consequently, improving cost efficiency.

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