Stability of cetuximab and panitumumab in glass vials and polyvinyl chloride bags

Hiroaki Ikesue, Lee C. Vermeulen, Rita Hoke, and Jill M. Kolesar

Cetuximab (Erbitux, Bristol-Myers Squibb) is an immunoglobulin G₁ human–mouse chimerized monoclonal antibody directed against the epidermal growth factor receptor (EGFR). It is indicated for the treatment of head and neck cancer and EGFR-expressing metastatic colorectal cancer. The drug is commercially available in single-use vials of 100 mg/50 mL and 200 mg/100 mL. The labeled dosage is 400 mg/m² as the initial loading dose, followed by weekly doses of 250 mg/m², administered as undiluted drug added to an empty infusion bag. Dosage reductions to 150–200 mg/m² per week are recommended for patients developing severe acne-like rashes (grade III or IV). The manufacturer recommends that any unused portion of the vial should be discarded and that preparations injected into infusion bags should be used within 12 hours if stored at 2–8 °C.

Panitumumab (Vectibix, Amgen) is an immune globulin G₂ fully human monoclonal antibody against the EGFR. It is indicated for the treatment of EGFR-expressing metastatic colorectal cancer. The drug is commercially available in single-use vials of 100 mg/5 mL, 200 mg/10 mL, and 400 mg/20 mL. The dosage is 6 mg/kg every 14 days. Doses

Purpose. The stability of cetuximab and panitumumab in glass vials and polyvinyl chloride (PVC) bags stored at 4 °C for up to 14 days was studied.

Methods. Sixty milliliters of cetuximab was drawn directly from a commercially available vial, and 20 mL was injected into each of three sterile, empty, 100-mL PVC bags through a preattached 0.22-μm filter. Three milliliters of panitumumab and 21 mL of 0.9% sodium chloride injection were mixed, and 8 mL of the mixture was injected into each of three empty PVC bags through a preattached 0.22-μm filter. Samples were analyzed immediately after preparation and again after storage at 4 °C for 7 and 14 days. Cetuximab and panitumumab concentrations were measured using a modification of a previously described enzyme-linked immunosorbent assay method. Stability was defined as the mean concentrations of the test solutions being within assay variability of the initial concentration. Solution appearance and color were assessed by observing the samples against black and white backgrounds.

Results. The percentages of initial concentration of cetuximab and panitumumab were over 90% stored at 4 °C after 14 days. No changes in color or turbidity were observed in any of the vials and the prepared solutions.

Conclusion. Cetuximab 2 mg/mL was stable when stored for 14 days in a glass vial and in PVC bags at 4 °C. Panitumumab 20 mg/mL in a glass vial and 2.5 mg/mL in 0.9% sodium chloride injection in PVC bags was also stable when stored for 14 days at 4 °C.

Index terms: Antibodies; Cetuximab; Concentration; Containers; Diluents; Glass; Immunoassay; Injections; Panitumumab; Polyvinyl chloride; Sodium chloride; Stability; Storage

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of up to 1000 mg are diluted to a total volume of 100 mL in 0.9% sodium chloride injection, and doses greater than 1000 mg are diluted to a total volume of 150 mL; the drug concentration should not exceed 10 mg/mL. A representative drug concentration for a small patient would be 2.5 mg/mL. The manufacturer recommends that any unused portion of the vial contents be discarded and that diluted infusion solutions of panitumumab should be used within 24 hours of dilution if stored at 2–8 °C.

The short stability of cetuximab and panitumumab results in significant waste, as vials are single use, and prepared solutions cannot be made in batches. The purpose of this study was to evaluate the stability of cetuximab and panitumumab in vials and polyvinyl chloride (PVC) bags at 4 °C for up to 14 days.

Methods
Sample preparation. Sixty milliliters of cetuximab was drawn directly from a commercially available vial, and 20 mL was injected into each of three sterile, empty, 100-mL PVC bags through a preattached 0.22-μm filter using aseptic technique. Three milliliters of panitumumab and 21 mL of 0.9% sodium chloride injection were mixed, and 8 mL of the mixture was injected into each of three sterile, empty PVC bags through a preattached 0.22-μm filter using aseptic technique to a final concentration of 2.5 mg/mL. Samples were analyzed immediately after preparation and again after storage at 4 °C for 7 and 14 days.

The preattached 0.22-μm filter is intended to maintain sterility during production. Its use has been shown to maintain sterility in media-fill validation studies under worst-case conditions. To evaluate the risk of drug adsorption to the filter, binding of the drug to the bag–filter assembly used in preparation was evaluated on day 0. Drug concentrations were measured in duplicate for each of the vials and PVC bags.

Enzyme-linked immunosorbent assays. On days 0, 7, and 14, 10-μL samples were removed from vials and bags. The cetuximab concentration of each sample was measured using a modification of a previously described enzyme-linked immunosorbent assay (ELISA) method. One hundred microliters of recombinant human EGFR (1 μg/mL) was added to each receptacle of 96-well microtiter plates with 50 mM sodium carbonate buffer (pH 9.6) and incubated overnight at 4 °C. The plates were washed three times with wash buffer (10 mM phosphate-buffered saline [PBS], pH 7.4, containing 0.05% polysorbate 20). To reduce background interference caused by nonspecific binding of cetuximab and panitumumab to surfaces of plastic ELISA plates, each well was then blocked with 200 μL of 1% bovine serum albumin (BSA) in PBS for one hour at room temperature. After blocking, plates were washed again three times with wash buffer. Subsequently, 100-μL portions of standards or samples were added to duplicate wells and incubated for two hours at room temperature with mild agitation. Standards and samples were diluted with ELISA diluent (1% BSA in PBS containing 0.05% polysorbate 20) before the assay procedure. Standard drug concentrations were 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, and 2.50 ng/mL.

Samples of cetuximab were diluted 1:3,200,000. After two hours of incubation, the plates were washed six times with wash buffer, and 100 μL of horseradish peroxidase (HRP)-conjugated AffiniPure rabbit anti-human immune globulin, Fcy fragment, 1:10,000 diluted with ELISA diluent, was added to each well. After two hours of incubation at room temperature, the plates were washed six times in ELISA wash buffer. One hundred microliters of 3,3′,5,5′-tetramethylbenzidine substrate solution was added to each well, and the reaction was developed at room temperature for 20 minutes while protected from light. The color reaction was stopped by adding 100 μL of 2 N sulfuric acid per well. The absorbencies of the well contents were read at 450 nm minus the 540-nm reference absorbance using an automatic plate reader.

The cetuximab standard curve was linear from 0.16 to 2.50 ng/mL. The intraday and interday coefficients of variation were <11.7% and <15.1%, respectively. Correlation coefficient values for the linear range of the dilution curves were 0.99 for day 0, 0.98 for day 7, and 0.97 for day 14.

Panitumumab concentrations were measured as above. Samples from the vial were diluted 1:32,000,000 with ELISA diluent, and samples from PVC bags were diluted 1:4,000,000. HRP-conjugated AffiniPure rabbit anti-human immunoglobulin G, Fcy fragment, was diluted 1:50,000 with ELISA diluent.

The panitumumab standard curve was linear from 0.08 to 2.50 ng/mL. The intraday and interday coefficients of variation were <4.5% and <12.1%, respectively. Correlation coefficient values for the linear range of the dilution curves were 0.98 for day 0, 0.99 for day 7, and 0.99 for day 14.

Stability was assessed by measuring the percentage of the initial concentration remaining at the end of each time interval. Stability was defined as the mean concentrations of the test solutions being within assay variability or within 15% (85–115%) of the initial concentration. Physical stability was assessed by visual inspection against black and white backgrounds.

Results
The percentages remaining of the initial concentrations of cetuximab and panitumumab stored at 4 °C for 14 days exceeded 90% (Table 1). No changes in color or turbidity were

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**Table 1.** The percentages remaining of the initial concentrations of cetuximab and panitumumab stored at 4 °C for 14 days exceeded 90%.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percentage Remaining (%)</th>
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<tbody>
<tr>
<td>Cetuximab</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>&gt; 90</td>
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</tbody>
</table>

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observed in any of the vials or the prepared solutions.

On day 0, there was no change in concentration of cetuximab and panitumumab before or after filtering, demonstrating no measurable drug adsorption to the filter when using the described system to compound the samples.

Discussion

Cetuximab and panitumumab were stable for 14 days at 4 °C, suggesting that the use of solutions for multiple doses or the preparation of stock solutions—if refrigerated and administered within 14 days—is a viable option to reduce drug costs.

The concentrations of the cetuximab vial at day 7 and of the panitumumab vial at day 14 were higher than expected, though these concentrations were within assay variability. These results are limited by the use of only one vial in the experiment. Due to the cost of the vials (cetuximab average wholesale price [AWP], $576.00 per 100-mg vial; panitumumab AWP, $1018.50 per 100-mg vial), only one vial of each drug was assayed.

Based on definitions from United States Pharmacopeia chapter 797 on pharmaceutical compounding and sterile preparations, the "expiration date" of medications is differentiated from the "beyond-use date." The expiration date is assigned by the manufacturer and shown on the product label. The beyond-use date is assigned by the pharmacy after compounding the product. Data on the stability of a compounded sterile product can be taken from the package insert for the product, but the beyond-use date can also be set using data from other references, including published stability studies. Our data provide evidence to support beyond-use dating longer than that provided in the package inserts for cetuximab and panitumumab. Lengthening the beyond-use date can improve efficiency of care delivery by allowing dose preparation well in advance of administration (reducing treatment delays and improving patient satisfaction) and can reduce economic losses due to waste (i.e., fewer doses not administered will pass the beyond-use date and be discarded).

The effect on cost may be substantial, as the AWP of cetuximab is $5.76/mg and panitumumab is $10.19/mg. The effect on cost may be substantial, as the AWP of cetuximab is $5.76/mg and panitumumab is $10.19/mg. None of the samples were tested for sterility.

Conclusion

Cetuximab 2 mg/mL was stable when stored for 14 days in a glass vial and in PVC bags at 4 °C. Panitumumab 20 mg/mL in a glass vial and 2.5 mg/mL in 0.9% sodium chloride injection in PVC bags was also stable when stored for 14 days at 4 °C.

Table 1. Stability of Cetuximab and Panitumumab in Manufacturers’ Vials and in Infusion Bags and Effect of Filtration on Concentration

<table>
<thead>
<tr>
<th>Drug and Container</th>
<th>Mean (Duplicate Values) or Mean ± S.D. Initial Concentration (mg/mL)</th>
<th>Mean (Duplicate Values) or Mean ± S.D. % Initial Concentration Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Cetuximabb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vial (n = 1)</td>
<td>2.00 (1.89, 2.11)</td>
<td>104.8 (94.0, 115.6)</td>
</tr>
<tr>
<td>PVC bag (n = 3)</td>
<td>2.04 ± 0.07</td>
<td>95.7 ± 7.3</td>
</tr>
<tr>
<td>Panitumumabc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vial (n = 1)</td>
<td>20.00 (19.30, 20.70)</td>
<td>97.3 (96.8, 97.8)</td>
</tr>
<tr>
<td>PVC bag (n = 3)</td>
<td>2.55 ± 0.18</td>
<td>103.3 ± 3.0</td>
</tr>
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</table>

*aSamples were assayed in duplicate. For bags, duplicate values were averaged, and the three averages were used to compute S.D. Drugs were filtered during injection into bags, so the initial concentrations in vials and bags were measured to assess the effect of filtration on drug concentration. PVC = polyvinyl chloride.

bNominal concentration, 2 mg/mL.

bNominal concentration, 20 mg/ml in the vial and 2.5 mg/ml in bags.
References

8. Red Book Update. 2009 (Sep); 29:34,66.