Chemical Stability of Admixtures Containing Ziconotide 25 mcg/mL and Morphine Sulfate 10 mg/mL or 20 mg/mL During Simulated Intrathecal Administration

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ABSTRACT

The chemical stability of an intrathecally administered analgesic combination may influence the frequency of pump refills necessary to maintain safe and effective analgesia. Previous work has shown that the stability of ziconotide at body temperature is reduced substantially by the presence of morphine sulfate 35 mg/mL. The current study was performed to evaluate the chemical stability of admixtures combining ziconotide with lower concentrations of morphine sulfate during simulated intrathecal infusion under laboratory conditions at 37°C. Admixtures containing ziconotide 25 mcg/mL and morphine sulfate 10 mg/mL or 20 mg/mL were stored in implantable intrathecal pumps at 37°C and in control vials at 37°C or 5°C. Samples were obtained over 60 days (admixture containing morphine sulfate 10 mg/mL) or 28 days (admixture containing morphine sulfate 20 mg/mL) and drug concentrations were assessed by high-performance liquid chromatography. Estimates of the time intervals that each admixture retained ≥90% and ≥80% of the initial concentrations of both drugs (i.e., the 90% and 80% stabilities) were based on 95% confidence bounds obtained via linear regression. Morphine was stable in both admixtures. In the admixture containing morphine sulfate 10 mg/mL, the mean ziconotide concentration declined to 81.4% of the initial concentration in 60 days, and 90% and 80% stabilities were maintained for 34 days and 65 days, respectively. In the admixture containing morphine sulfate 20 mg/mL, the mean ziconotide concentration declined to 85.3% of the initial concentration in 28 days, and 90% and 80% stabilities were maintained for 19 days and 37 days, respectively. Decreasing the concentration of morphine in an admixture containing ziconotide improves the stability of ziconotide.

INTRODUCTION

Ziconotide is the synthetic equivalent of a naturally occurring conopeptide isolated from the venom of the marine snail *Conus magus*.[1] In 2004, the U.S. Food and Drug Administration approved ziconotide for the management of severe chronic pain in patients for whom intrathecal therapy is warranted and who are intolerant of or whose pain is refractory to other treatments such as systemic analgesics, adjunctive therapies, or intrathecal morphine.[1] Ziconotide monotherapy is recommended as a first-line intrathecal therapy option by the 2007 Polyanalgesic Consensus Conference (PACC) panel members.[2]

For patients who do not receive satisfactory pain control from a single intrathecal agent, intrathecal administration of a combination of analgesics is an accepted treatment approach.[2,3] In theory, patients are most likely to benefit from combination therapy if the combined drugs have different mechanisms of action. Research in animals suggests that ziconotide inhibits neurotransmission from primary nociceptive afferents in the dorsal horn of the spinal cord by binding to and directly blocking N-type voltage-sensitive calcium channels. Morphine administration also inhibits N-type voltage-sensitive calcium channels, but the mechanism is modulated indirectly through opioid receptors, and morphine exerts additional effects on the activity of postsynaptic potassium channels.[4,5] Thus, the different analgesic mechanisms employed by ziconotide and morphine may yield additive effects when delivered as an intrathecal combination. This theory is supported by two recent open-label studies that revealed that combination therapy with intrathecal morphine and ziconotide produced greater pain relief than therapy with either analgesic alone.[6,7] Combination of ziconotide with morphine is recommended as a second-line intrathecal treatment option by the 2007 PACC panel members.[2]

The chemical stability of an analgesic combination is an important consideration when choosing medications because less stable combinations could result in more frequent pump refills and could compromise the accuracy of the dose delivered. Previous work has shown that the stability of ziconotide at body temperature is reduced substantially by the presence of morphine sulfate,[8] but that study used a higher morphine concentration (35 mg/mL) than is recommended in guidelines developed by the 2007 PACC panel (20 mg/mL).[3] The current study was performed to evaluate the chemical stability of admixtures containing ziconotide 25 mcg/mL and morphine sulfate 10 mg/mL or 20 mg/mL under simulated clinical conditions.

MATERIALS AND METHODS

Admixture Preparation

This study consisted of two experiments. In the first experiment, ziconotide (Lot

CM2-345; PRIALT, ziconotide intrathecal infusion, Elan Pharmaceuticals, Inc., South San Francisco, California) was obtained in 20-mL vials as a sterile, pyrogen-free solution containing ziconotide 25 mcg/mL, methionine 50 mcg/mL (to reduce ziconotide oxidation), and sodium chloride 9 mg/mL. The pH was adjusted to between 4.0 and 5.0. Aseptic technique was used to pool 200 mL of ziconotide solution into a sterile plastic bottle, and 2 g of lyophilized morphine sulfate (Lot 6116-PMS50002; B&B Pharmaceuticals, Inc., Aurora, Colorado) was added to the pooled solution for final drug concentrations of ziconotide 25 mcg/mL and morphine sulfate 10 mg/mL. In the second experiment, a similar technique was used; 4 g of lyophilized morphine sulfate was added to the pooled ziconotide solution (Lot CM2-343; Elan Pharmaceuticals, Inc.) to achieve final drug concentrations of ziconotide 25 mcg/mL and morphine sulfate 20 mg/mL.

Because the primary ziconotide degradation pathway is oxidative, preparative techniques that minimize the presence of dissolved oxygen in the admixtures were used. Dissolution of morphine sulfate was facilitated with gentle shaking to minimize the introduction of atmospheric oxygen into the admixtures. After the morphine sulfate dissolved fully, the admixtures were sparged by bubbling nitrogen through the solution to remove dissolved oxygen.

Pump Preparation and Fill

In both experiments, the same pump preparation, filling, sampling, and analytical procedures were used, except as noted.

After nitrogen sparging, the admixture and SynchroMed II pumps (Model 8637-20; Medtronic Inc., Minneapolis, Minnesota) that had been exposed previously to ziconotide were warmed for 1 hour in a calibrated 37°C incubator. Each pump septum was wiped with an alcohol swab to prevent bacterial contamination, and then residual fluid was removed from the pump reservoirs through the pump septa with a 23-gauge needle attached to a sterile 20-mL syringe. The fluid was drawn aseptically into the syringe and discarded.

Pump reservoirs were rinsed twice with approximately 5 mL of the admixture solution to ensure that drug concentrations were not affected by any residual fluid left in the pump reservoir. A sterile 20-mL syringe was filled with 10 mL of admixture solution and attached to a 0.22-mcm filter and 23-gauge needle. Air was removed from the syringe, and the filter and needle were flushed with 1 mL of admixture solution. After the pump septum was wiped with an alcohol swab, the needle was inserted through the pump septum and the reservoir was filled aseptically with approximately 5 mL of the admixture solution. The pump was shaken gently to distribute the solution throughout the reservoir, then the admixture was removed aseptically from the reservoir through the pump septum with another needle attached to a clean syringe. The pump was then filled with the remaining admixture in the filter-equipped syringe and programmed to deliver the admixture at the maximum possible rate in order to precondition the pump's fluid path. The admixture was delivered to waste for 30 minutes, then delivery was stopped, and all remaining admixture solution was removed from the reservoir by using a clean syringe and needle.

Pumps were then filled aseptically with 20 mL of the admixture solution by using a new filter-equipped syringe. Controls were established at the beginning of the study by aseptically filling polymethylpentene high-performance liquid chromatography (HPLC) vials to capacity with 750 mcL of admixture by using a needle and syringe. Because intrathecal pumps are implanted in clinical use, all pumps and half the control vials were stored in the 37°C incubator during the study, except for the brief period when the pumps were removed from the incubator to attach the catheter before sampling. The other half of the control vials were stored in a refrigerator at 5°C.

Sampling

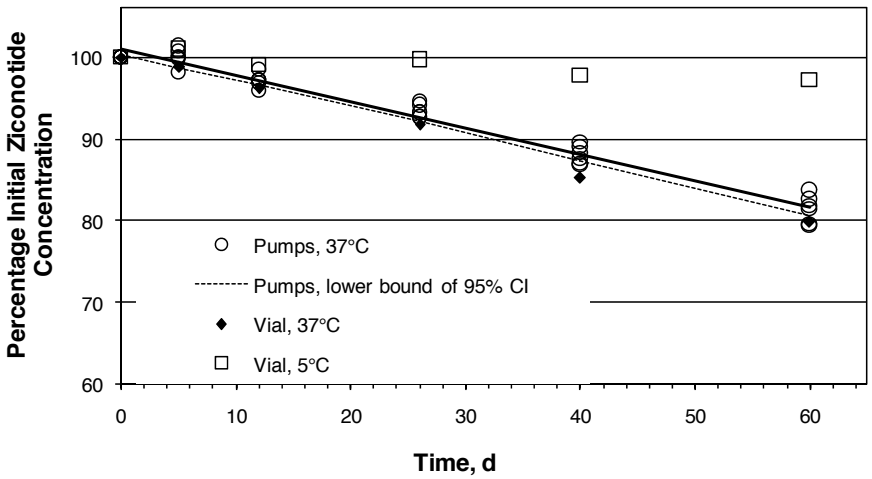
Because of the limited number of samples obtainable from each pump, the sampling schedules were determined while the study was ongoing. Pumps and control vials were sampled within minutes of filling (day 0) and on days 5, 12, 26, 40, and 60 (admixture containing morphine sulfate 10 mg/mL) or on days 0, 6, 13, 21, and 28 (admixture containing morphine sulfate 20 mg/mL). Before sample collection, the pump's fluid path was equilibrated with the reservoir contents by delivering approximately 1.5 mL of admixture solution into a waste vial. Each pump's catheter port cap was removed, and the catheter port was swabbed with sterile 70% isopropyl alcohol. A 40-cm length of catheter, with a catheter connector pin pushed into the distal end, was attached to the pump's catheter port, and a waste vial was sealed to the catheter by pushing the catheter connector pin through the vial septum. A vent, composed of a 0.22-mcm sterile syringe filter and a 27-gauge needle, was provided to release headspace backpressure as the vial filled. The pump was programmed to deliver at the maximum infusion rate and returned to the 37°C incubator until a sufficient amount of solution had been delivered into the vial. Delivery was stopped, and the waste vial was replaced with a vented sample collection vial. The pump was again programmed to deliver at the maximum rate and placed in the incubator at 37°C until approximately 0.5 mL of the admixture had been collected in the sample vial. The catheter was detached, and the remaining catheter solution was pushed into the collection vial using a nitrogen-filled syringe. When sampling was complete, the pump was programmed to deliver at the minimum rate and stored in the 37°C incubator. The sample collection vials, along with one control vial each from the 37°C and 5°C storage conditions, were transferred to the HPLC apparatus for analysis.

Analytical Equipment and Conditions

Ziconotide and morphine sulfate concentrations were measured with an Agilent Series 1100 HPLC system (Agilent Technologies, Inc., Santa Clara, California), or equivalent, equipped with a Phenomenex Jupiter, 300-Å pore size, C18, 5-mcm, 250 × 4.6-mm column (Phenomenex, Inc., Torrance, California) maintained at 40°C as the stationary phase. The mobile phase consisted of gradient elution starting with 100% mobile phase A for 2 minutes, transitioning to 70% mobile phase A:30% mobile phase B over 12 minutes, then to 30% mobile phase A:70% mobile phase B over the next 10 minutes, before returning to 100% mobile phase A for approximately 6 minutes to re-equilibrate the column. The flow rate used throughout the 30-minute procedure was 2.0 mL/min. Mobile phase A consisted of 98% 25 mmol/L sodium perchlorate, 2% methanol, and 0.05% trifluoroacetic acid; mobile phase B consisted of 49.95% acetonitrile, 49.95% water, and 0.1% trifluoroacetic acid. The ultraviolet detector was set at 212 nm. Because of the nearly 1000-fold difference between the concentrations of morphine sulfate and ziconotide, each sample solution was injected twice.

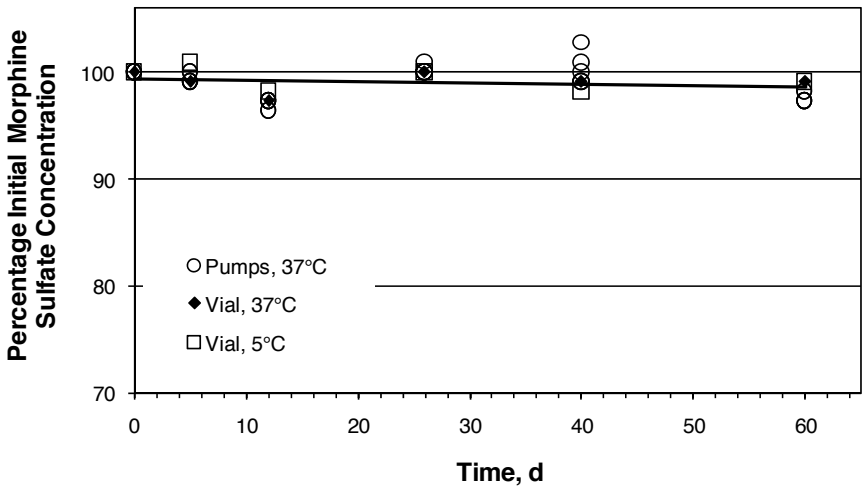
FIGURE 1. The stability of an admixture containing ziconotide 25 mcg/mL and morphine sulfate 10 mg/mL.

FIGURE 1A



Ziconotide concentrations. The concentrations of ziconotide measured in replicate implantable intrathecal pumps (open circles) stored at 37°C, and in vials stored at 37°C (diamonds) or 5°C (open squares), are expressed as percentages of the concentration measured at study initiation (day 0). A linear least-squares fit is displayed for the available pump data (solid line), and the lower bound of the 95% confidence interval (CI; dotted line) is also provided.

FIGURE 1B



Morphine sulfate concentrations. The concentrations of morphine sulfate measured in replicate implantable intrathecal pumps (open circles) stored at 37°C, and in vials stored at 37°C (diamonds) or 5°C (open squares), are expressed as percentages of the concentration measured at study initiation (day 0). A linear least-squares fit (solid line) of the pump data is displayed.

For ziconotide analysis, an injection volume of 150 mcL was used; 1 mcL was used for morphine sulfate analysis. A qualification was performed on this method, which included stability, range/linearity, accuracy/recovery, specificity, and precision; all met predefined acceptance criteria.

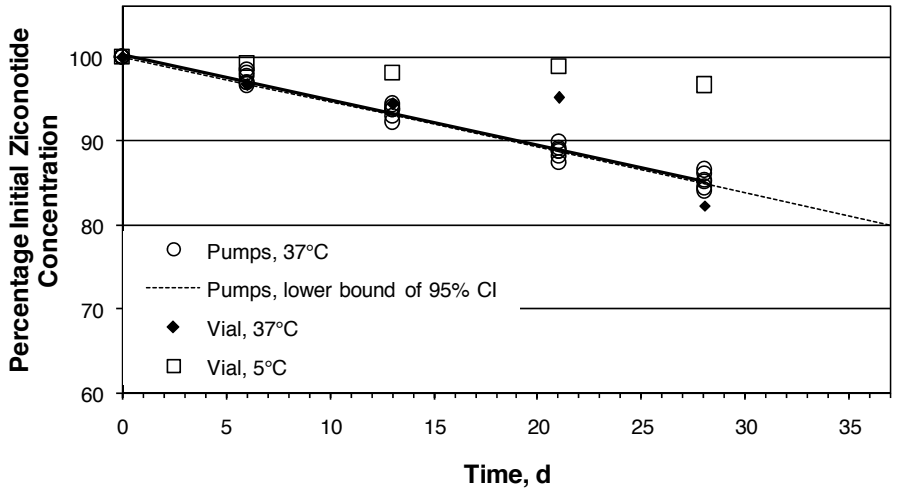
The solution stability data indicated that the admixture of ziconotide and morphine sulfate must be assayed within 10 days of sampling to prevent additional degradation. Linearity and range were established for ziconotide between 80% and 400% of standard concentration (25 mcg/mL). Linearity for morphine sulfate was established between 80% and 110% of standard concentration (35 mg/mL). The correlation coefficients of the linear regressions for each of the linearity series were within the acceptance criteria, with correlation coefficients of 1.000 and 0.997 for ziconotide and morphine sulfate, respectively. All results used to calculate linearity were within 3% of the results expected from the determined regression line. Accuracy (± 5%) was demonstrated for admixtures of ziconotide and morphine sulfate tested within 80% to 110% of specified concentrations. Specificity was demonstrated, with both major peaks having a resolution of greater than 1.5. Precision was determined across instruments, analysts, and days. The results indicated that there was no significant difference in either ziconotide or morphine sulfate results (<2.5%) when instrument, analyst, or day was varied.

Statistics

For each pump, the concentrations of ziconotide and morphine sulfate on each sampling day were converted to a percentage of that measured on day 0. Statistical software package SAS version 8.2 (SAS Institute Inc., Cary, North Carolina) was used to conduct a linear regression analysis to predict the time dependence of the percentage of ziconotide remaining in the pumps and the corresponding 95% confidence interval (CI). Admixture stability is reported as the number of days that the lower bound of the 95% CI for concentrations of both drugs remained at or above 90% (90% stability) or 80% (80% stability) of the initial concentrations. Values that exceed the duration of the study (i.e., 60 days for the first experiment or 28 days for the second experiment) were extrapolated, assuming linear degradation.

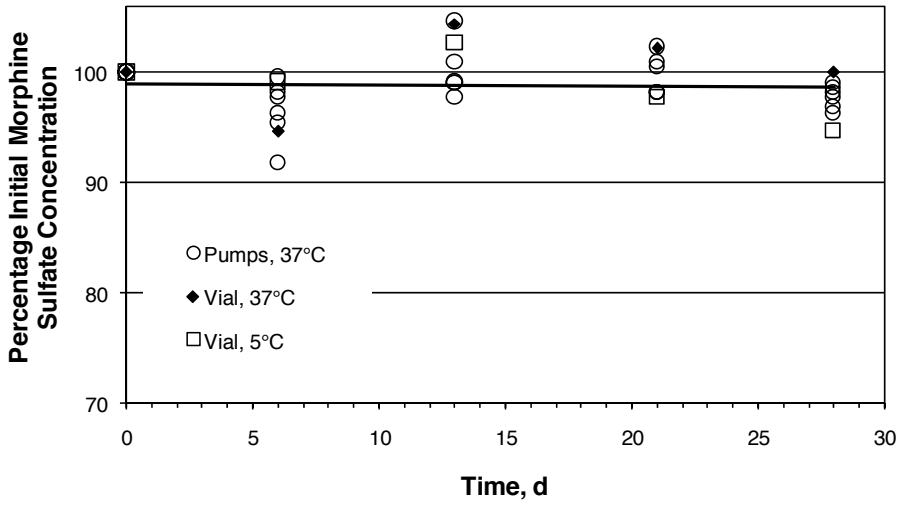
FIGURE 2. The stability of an admixture containing ziconotide 25 mcg/mL and morphine sulfate 20 mg/mL.

FIGURE 2A



Ziconotide concentrations. The concentrations of ziconotide measured in replicate implantable intrathecal pumps (open circles) stored at 37°C, and in vials stored at 37°C (diamonds) or 5°C (open squares), are expressed as percentages of the concentration measured at study initiation (day 0). A linear least-squares fit is displayed for the available pump data (solid line), and the lower bound of the 95% confidence interval (CI; dotted line) is also provided.

FIGURE 2B



Morphine sulfate concentrations. The concentrations of morphine sulfate measured in replicate implantable intrathecal pumps (open circles) stored at 37°C, and in vials stored at 37°C (diamonds) or 5°C (open squares), are expressed as percentages of the concentration measured at study initiation (day 0). A linear least-squares fit (solid line) of the pump data is displayed.

RESULTS

Morphine Sulfate 10-mg/mL and Ziconotide 25-mcg/mL Admixture

The mean concentration of ziconotide was 97.1% of the initial concentration on day 12 and 81.4% of the initial concentration on day 60 (Figure 1A); the standard deviation (SD) was no more than 1.7% on any sampling day. The lower bound of the 95% CI for ziconotide concentration remained above 90% and 80% of the initial ziconotide concentration for 34 days and 65 days, respectively. A similar rate of decay was observed in the control vials stored at 37°C; the concentration of ziconotide fell to 96.2% of initial concentration on day 12 and 79.8% of initial concentration on day 60. Control vials stored at 5°C maintained 97.1% of the initial ziconotide concentration on day 60.

Concentrations of morphine sulfate in pump samples averaged 97.0% of the initial concentration on day 12 and 97.6% of the initial concentration on day 60 (Figure 1B); variability in pump measurements was ≤1.4% SD at all time points. The lower bound of the 95% CI for morphine sulfate concentration remained above 90% of the initial concentration for the duration of the study. In control vials stored at either 37°C or 5°C, the concentrations of morphine sulfate remained at or near 100% of the initial concentration throughout the study.

Morphine Sulfate 20-mg/mL and Ziconotide 25-mcg/mL Admixture

The concentration of ziconotide in pump samples averaged 93.5% of the initial concentration on day 13 and 85.3% of the initial concentration on day 28 (Figure 2A); variability was no greater than 0.9% SD on any sampling day. The lower bound of the 95% CI for ziconotide concentration remained above 90% and 80% of the initial ziconotide concentration for 19 days and 37 days, respectively. Ziconotide concentrations in the control vials stored at 37°C displayed a similar rate of decay, falling to 94.5% of the initial concentration on day 13 and to 82.4% of the initial concentration on day 28. Control vials stored at 5°C retained 96.7% of the initial ziconotide concentration on day 28.

Morphine sulfate concentrations in pump samples averaged 100.1% of the initial concentration on day 13 and 97.8% of the initial concentration on day 28 (Figure 2B);

variability in pump measurements was ≤ 2.7% SD at all time points. The lower bound of the 95% CI for morphine sulfate concentration remained well above 90% of the initial morphine sulfate concentration for the duration of the study. The concentrations of morphine sulfate in control vials stored at either 37°C or 5°C remained at or near 100% of the initial concentration throughout the study.

DISCUSSION

Morphine is the most commonly prescribed intrathecal analgesic for chronic pain management, and most physicians who use implantable infusion pumps have prescribed intrathecal therapy that combines morphine with another intrathecally administered drug.[9] Evidence from animal models and two recent open-label studies suggest that simultaneous intrathecal administration of morphine and ziconotide produces additive analgesia,[6,7,10] but high concentrations of morphine sulfate have been shown to reduce ziconotide stability,[8] which may influence the clinical utility of this analgesic combination.

A previous study investigated the stability of an admixture containing ziconotide 25 mcg/mL and morphine sulfate 35 mg/mL by using the same protocol and statistical analyses described here.[8] Morphine sulfate was stable under all conditions for the duration of this earlier study (17 days), whereas ziconotide concentrations declined to 90% of the initial concentration in 8 days and to 80% of the initial concentration in 15 days. However, the morphine sulfate concentration used in the study exceeded the maximum concentration recommended by the 2007 PACC panel members (20 mg/mL).[2] In the current study, the stability of admixtures containing ziconotide and lower concentrations of morphine sulfate was investigated.

Results of the present study reveal that the stability of ziconotide in admixtures improves substantially when lower concentrations of morphine sulfate are used. In the admixture containing morphine sulfate 10 mg/mL, 90% stability and 80% stability were maintained for 34 days and 65 days, respectively; in the admixture containing morphine sulfate 20 mg/mL, 90% stability and 80% stability were maintained for 19 days and 37 days, respectively. Taken together, these results demonstrate that when ziconotide and morphine sulfate are compounded for intrathecal administration, the stability of ziconotide is strongly dependent on the concentration of morphine sulfate. The clinical implications of these findings are limited by the *in vitro* nature of the studies.

In both admixtures, ziconotide and morphine sulfate were stable when stored in vials at 5°C. These results suggest that little, if any, decline in concentration will occur during refrigerated storage and shipment.

Identification of the degradation products in these admixtures was beyond the scope of this investigation. When ziconotide alone is stored in implantable pumps, the primary ziconotide degradation product is produced when oxygen reacts with the peptide methionine (data on file, Elan Pharmaceuticals, Inc.); therefore, reducing the presence of dissolved oxygen in admixtures should improve stability. For this reason, the admixtures in this study were prepared with lyophilized morphine sulfate, rather than with a commercially available formulation that may contain dissolved oxygen. Oxygen is removed from commercial ziconotide formulations by nitrogen sparging during manufacture; the additional sparging performed during preparation of the admixtures for this investigation served primarily to remove oxygen that may have entered the solution during compounding. Omitting this step may speed ziconotide degradation and reduce admixture stability.

CONCLUSION

This report characterized the long-term chemical stability of admixtures that paired ziconotide 25 mcg/mL with morphine sulfate 10 mg/mL or 20 mg/mL under conditions that simulated clinical use. When stored in implantable pumps at 37°C, the admixture containing morphine sulfate 10 mg/mL retained at least 90% of the initial ziconotide concentration for 34 days and 80% of the initial ziconotide concentration for 65 days, and the admixture containing morphine sulfate 20 mg/mL retained at least 90% of the initial ziconotide concentration for 19 days and 80% of the initial ziconotide concentration for 37 days. Minimal ziconotide loss was observed in refrigerated vials, and morphine sulfate concentrations were stable under all conditions. Clinical trials are necessary to evaluate the efficacy and safety of this drug combination.

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