Stability of Parenteral Midazolam in an Oral Formulation

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Midazolam is increasingly being used for oral sedation in pediatric dentistry. Unfortunately, it is available only as a parenteral formulation in Canada and the United States. Preparation of the parenteral solution for oral use is not uniform and leads the clinician to question the stability of this drug when used in conjunction with these vehicles. Therefore, the purpose of this study was to investigate the chemical stability of parenteral midazolam as an oral formulation to determine its expiry date. This was evaluated using a validated stability-indicating liquid chromatographic method. Midazolam was diluted in orange-flavored syrup to yield concentrations of 0.35, 0.64, and 1.03 mg/ml and then stored at room temperature. Samples were drawn on each of 9 study days (0, 1, 2, 6, 7, 9, 13, 21, and 102) and chromatographed. On each study day, solutions were inspected visually for changes in color, clarity, and appearance of particulate matter. Midazolam concentrations were considered within acceptable limits if they were not less than 90% of the initial concentration. Over the 102-day study period, there was no significant change in concentration in any of the solutions. On day 102, the remaining midazolam was within 7% of the day zero concentration. Therefore, these formulations of midazolam are stable at room temperature for a period of 102 days and would be suitable for clinical use.

Key Words: Midazolam; Stability; Oral formulation.

Midazolam, the short-acting water-soluble benzodiazepine most commonly used for intravenous conscious or deep sedation, is now being used as an oral sedative for children prior to the administration of general anesthetics.[1] Most recently, it is also being used for oral sedation of conscious patients in dentistry.[2] Unfortunately, it is available only as a parenteral formulation in Canada and the United States. This formulation has a very bitter taste, and therefore, if it is to be administered orally, it must have this taste disguised. This has resulted in a great variety of clinician-prepared solutions where the parenteral formulation has been dissolved in a variety of vehicles. The dilution of midazolam into these vehicles is not standardized and leads the clinician to question the potential degradation of the active drug. Therefore, the purpose of this study was to investigate the chemical stability of parenteral midazolam in an orange-flavored vehicle to determine a reasonable expiry date.

Previous studies have shown that intravenous solutions of midazolam will retain more than 90% of the initial concentration when stored in normal saline or 5% dextrose in water solutions for up to 30 days at room temperature (23°C) or at 4°C.[3] Intravenous solutions of midazolam are compatible with either morphine or haloperidol in 5% dextrose in water solutions for 14 days at room temperature[4] and, when stored in polypropylene syringes, for 7 days.[5] In parenteral nutrition solutions, midazolam is reported to be stable for only 5 hr,[6] although this study only evaluated midazolam concentrations over a 5-hr period. Therefore, in intravenous solutions, midazolam appears to be relatively stable. However, studies of the stability of oral formulations

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Table 1. Vehicle Used for Dilution of Midazolam (5 mg/ml)

Ingredient	*Source*	*Volume*[a]
Simple syrup USP	Drug Trading Company, Toronto, Ontario, Canada	50 ml
Pure orange extract	Club House® McCormick Canada Inc.[b]	0.12 ml
Red food color	Club House® McCormick Canada Inc.[b]	1 drop
Yellow food color	Club House® McCormick Canada Inc.[b]	1 drop
Distilled water		to 100 ml

[a] Directions: add about 30 ml of water to 50 ml of simple syrup. Add 0.12 ml of orange extract flavoring. Shake and add 1 drop of each food color. Shake and then add distilled water to bring the total volume to 100 ml.
[b] These items were purchased from a local retail grocery store.

of midazolam have produced conflicting results. Some investigators have reported that a sweetened and/or flavored formulation is stable for 14 days in the refrigerator[7] or 56 days at temperatures up to 40°C,[8] while other investigators[9] have observed a 25% reduction in the midazolam concentration by day 38 in 2.5 mg/ml and 3.0 mg/ml oral solutions. Soy et al report an 11% loss in concentration after 60 to 73 days of storage.[10] Therefore, it was the objective of this study to evaluate the stability of midazolam in a sweetened, flavored, and colored oral solution.

METHODS

Assay Validation

Following the set up of the chromatographic system for midazolam according to that reported by Hagan et al,[3] the suitability of this method for use as a stability-indicating assay was tested by accelerating the degradation of midazolam. A total of 39.7 mg of midazolam, as a free base, was dissolved in 50 ml of distilled water. All of the free-base midazolam in this study was provided by Hoffman-LaRoche, Nutley, New Jersey (Lot 816072, 99.99% pure). The pH of 15 ml aliquots of this solution was adjusted to 2.63 and 4.22 with 1 N HCl, and 9.80 and 12.16 with 0.1 N NaOH. A fifth aliquot was also used without pH adjustment (pH = 5.7). Each aliquot was placed in a glass vial, incubated in a water bath at 80°C, and protected from light for up to 50 hr. Samples were drawn from each solution just prior to incubation at 80°C and at least eight other times during the study period. Chromatograms were inspected for the appearance of additional peaks, and the midazolam peak was compared between samples for changes in concentration, retention time, and peak shape.

Following this first phase of evaluation and validation, the accuracy and reproducibility of standard curves were tested over 5 days and system-suitability criteria (theoretical plates, tailing, and retention time) were developed to ensure consistent chromatographic performance. On each day, 20 mg of midazolam powder, as a free base, was dissolved in 10 ml of a solution of 50% methanol and 50% distilled water. This stock solution of 2.0 mg/ml was then diluted to prepare six additional concentrations of 0.05, 0.10, 0.25, 0.50, 1.00, and 1.50 mg/ml. These seven samples plus a blank were used to construct a standard curve. Two additional samples prepared from a separate weighing of midazolam, as a free base, were dissolved in 10 ml of a solution of 50% methanol and 50% distilled water. These two samples (0.15 mg/ml and 0.05 mg/ml) were run daily and served as quality-control standards. Five microliters of each of these standards, the quality-control sample, and a blank were directly chromatographed in duplicate on 5 consecutive days.

Stability Study

On study day zero, 1, 2, or 3 ml of midazolam (Versed,® 5 mg/ml; Hoffmann-LaRoche) was diluted in 13 ml of orange-flavored syrup to yield concentrations of 0.35 mg/ml, 0.64 mg/ml, and 1.03 mg/ml (formulation provided in Table 1). Three samples of each concentration were prepared and all samples were stored in high-density polyethylene containers at room temperature (23 ± 2°C). Five μl of each sample were drawn on each of 9 study days (0, 1, 2, 6, 7, 9, 13, 21, and 102) and directly chromatographed in duplicate.

Midazolam Analysis

Standard curves were prepared daily by dissolving 20 mg of midazolam, as a free base, in 10 ml of a solution of 50% methanol and 50% distilled water. This stock solution of 2.0 mg/ml was then diluted to prepare six additional standard concentrations of 0.05, 0.10, 0.25, 0.50, 1.00, and 1.50 mg/ml. These seven samples plus a blank were used to construct a standard curve. Two additional samples prepared from a separate weighing of 15 mg of midazolam, as a free base, were dissolved in 10 ml of a solution of 50% methanol and 50% distilled water. These two samples were run daily and served as quality-control standards. Five microliters of

each of these standards, the quality-control sample, and a blank were directly chromatographed in duplicate.

The mobile phase was prepared by mixing a pH 7.0 phosphate buffer (prepared by combining 6.1 ml of 1.0 M dibasic potassium and 3.9 ml of 1.0 M monobasic potassium). Ten milliliters of this solution was diluted to 1.0 l with distilled water, filtered before use, and then mixed with the organic phase of methanol (OmniSolv; BDH Inc., Toronto, Ontario, Canada), acetonitrile (AX0142-1; Em Science, Gibbstown, New Jersey), and tetrahydrofuran (T425-1; Fisher Scientific, Toronto, Ontario, Canada). The mobile phase consisted of 56 parts of the phosphate buffer, 22 parts methanol, 22 parts acetonitrile, and one part tetrahydrofuran. The mobile phase is similar to that used by Hagan et al,[3] except that the percentage of the buffer was increased to prolong the retention time of midazolam and to allow the separation of midazolam from the coloring or flavoring used in the syrup. The liquid chromatographic (LC) system consisted of an isocratic solvent delivery pump (Model P100; Spectra Physics, San Jose, California), which delivered the mobile phase through a 25 cm × 4.2 mm reversed-phase C-18, 5 μm column (Ultrasphere ODS, 235329; Beckman, Mississauga, Ontario, Canada) at 1.0 ml/min. On each day the strength of the mobile phase was prepared to achieve a retention time for midazolam between 26 and 30 minutes. Samples were introduced into the LC system using an autoinjector (WISP 715; Waters Scientific, Toronto, Ontario, Canada). The column effluent was monitored with a variable wavelength ultraviolet detector (Model 1050; Hewlett Packard, Waldbronn, Germany) at 254 nm. The signal from the detector was integrated and recorded with a chromatographic integrator (Model 4240; Spectra Physics). The area under the midazolam peak at 254 nm was subjected to least squares linear regression and the actual midazolam concentration in each sample determined by interpolation from the standard curve. Midazolam concentrations were recorded to the nearest 0.01 mg/ml.

Physical Evaluation

On each study day, as each solution was drawn for determination of the midazolam concentration, solutions were inspected visually for changes in color and clarity and the appearance of particulate matter against a black-and-white background.

Data Reduction and Statistical Analysis

Means were calculated for analyses completed in duplicate and/or triplicate. Error was assessed by the coefficient of variation (CV). Mean results from different days of an identical test were compared statistically by least squares linear regression to determine whether or not an association existed between the observed result and time. Log-linear and linear-linear fits for the data from the accelerated degradation study (80°C) were compared for goodness of fit by the Maximum Likelihood Method of Box and Cox.[11,12] Analysis of variance and the least significant difference multiple range test were used to compare differences between concentrations on different days. The 5% level was used as the *a priori* cutoff for significance and all reference to significance refers to this level. Midazolam concentrations were considered "acceptable" or "within acceptable limits" if the concentration on any day of analysis was not less than 90% of the initial (day zero) concentration.

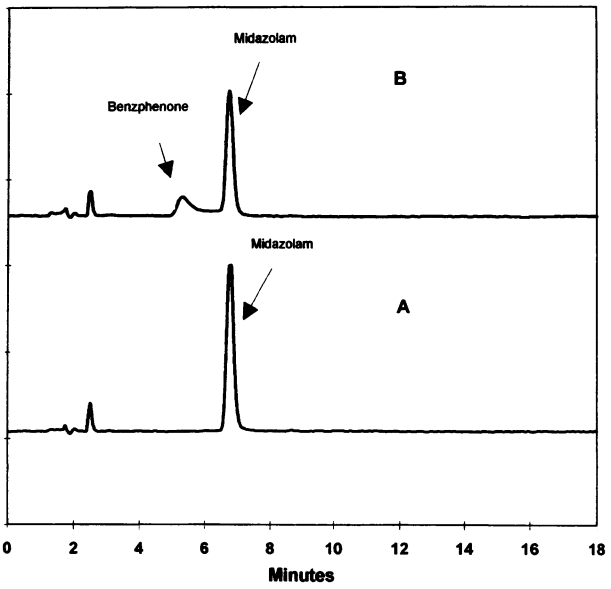

Figure 1. Chromatograms observed during the assay validation of midazolam. Samples of midazolam in distilled water (A) and following adjustment to a pH of 2.63 with 1N HCl (B). At a pH of 2.63, the sample drawn prior to incubation was observed to have a large peak that eluted just prior to midazolam (identified as benzophenone). This compound is in equilibrium with midazolam and is favored over the closed-ring structure of midazolam.[3,13,14]

RESULTS

Accelerated Degradation and Assay Validation

Chromatograms observed during the accelerated studies are shown in Figure 1. At a pH of 2.63, the sample drawn prior to incubation was observed to have a large peak that eluted just prior to midazolam (Figure 1B). At a pH of less than 3.3, an open-ring structure (benzophenone) is formed[3,13,14] from the midazolam. This compound is in equilibrium with and is favored over the

Table 2. Mean[a] Midazolam Concentration in Syrup Solutions

	Midazolam Concentration[b] (mg/ml)		
Study day	*0.35*	*0.64*	*1.03*
0	0.352 ± 0.004	0.642 ± 0.037	1.031 ± 0.040
1	0.331 ± 0.003	0.600 ± 0.031	1.005 ± 0.029
2	0.351 ± 0.006	0.625 ± 0.031	1.046 ± 0.041
6	0.357 ± 0.006	0.652 ± 0.004	1.074 ± 0.013
7	0.389 ± 0.008	0.704 ± 0.011	1.161 ± 0.012
9	0.367 ± 0.007	0.662 ± 0.002	1.075 ± 0.011
13	0.359 ± 0.002	0.635 ± 0.011	1.024 ± 0.016
21	0.352 ± 0.006	0.634 ± 0.010	1.057 ± 0.017
102	0.329 ± 0.006	0.606 ± 0.011	0.975 ± 0.006
Percent remaining—day 102[c]	97.30	94.94	95.23
Correlation coefficient (r)	−0.2999	−0.3748	−0.4935
P value ($P_{crit\ at\ 0.05} = 0.754$)	$P > 0.10$	$P > 0.10$	$P > 0.10$

[a] Mean concentrations are based on the average concentrations found in three solutions, each determined in duplicate. Mean concentrations are reported as the mean ± the standard deviation.

[b] Nominal concentration is calculated using known volumes of Versed® added and diluted with known volumes of orange-flavored syrup.

[c] Percent remaining on day 102 is calculated using least squares linear regression. Observed concentration on day zero is used as 100%, and day-102 concentration is calculated based on the slope determined by regression. Percent remaining is calculated based on the formula (concentration day 102) × 100/observed concentration day zero.

closed-ring form of midazolam[3,13,14] at these low pH values. The open-ring structure has been reported to interfere with midazolam and cannot be completely separated from midazolam; however, the open-ring structure is not a true degradation product, because it can completely revert to midazolam at a pH of 7.4[3,13,14]. At all other pHs, no significant degradation of midazolam was observed over a 50-hr period at 80°C. Over the 41.25-hr study period, there was less than 10% degradation and no additional peaks were observed in chromatograms. These conditions are more extreme than those reported by Hagan et al,[3] and although they indicated that one degradation product appeared in the solvent front after storage for 1 hr at room temperature, a similar peak was observed but did not change over the 41.25-hr study period. Nevertheless, the ability of the chromatographic system to separate midazolam from the open-ring benzophenone indicated that this analytical method was indicative of stability.[15,16]

Standard curves were linear up to 2 mg/ml and analysis of accuracy and reproducibility indicated that the midazolam concentration was measured accurately. Reproducibility, within a day (CV of replicates for each of the seven standards), averaged less than 3.5% on each day and less than 2% for each sample across all study days. Accuracy, based on the mean of duplicate determinations of the 0.17 mg/ml quality-control sample, was within 95 to 103% of the theoretical concentrations and the differences among days for the determination of the 0.17 mg/ml quality-control sample averaged 6.8%. This indicates that differences of 10% or more can be confidently detected with acceptable error rates.[17]

Midazolam Stability Study

Each of the nine orange-flavored midazolam solutions appeared as clear orange-colored liquids. Over the 102-day study period, there was no significant change in midazolam concentration in any solution at 23°C (Table 2), and on day 102, the percent remaining was within 3% of the day zero concentration. Because of the lack of degradation, concentration had no significant effect on the degradation rate and confidence estimates of the degradation rate could not be determined. All solutions remained clear and colorless throughout the duration of the 102-day study.

DISCUSSION

Statistical analysis of the midazolam concentration over time in this study was limited to least squares log-linear regression because demonstration of the decreasing concentration was considered more important than demonstrating a statistical difference in concentration between any 2 days. In fact, the random fluctuations in concentration around the initial concentration are not of any practical importance and should be considered "noise" or experimental error. Least squares log-linear

regression indicated that much less than a 6% loss in the initial midazolam concentration occurred during the 102-day study period at 23°C.

Because no change in midazolam concentration could be detected in any solution, assurance of the specificity of the analytical method is very important. The specificity of the analytical method was demonstrated during the accelerated degradation studies. At a pH of 2.63, a large peak that eluted just prior to midazolam was regarded as open-ring benzophenone.[13,14] This compound is in equilibrium with midazolam and is favored over the closed-ring structure of midazolam.[13,14] In this study, we were able to separate this compound from other constituents in the formulation and regarded the method as indicative of stability. The separation and detection of intact drug in the presence of degradation compounds must be assured before the method can be considered indicative of stability.[15,16]

Previous studies[3–6] have shown that intravenous solutions of midazolam will retain more than 90% of the initial concentration when stored in normal saline or 5% dextrose in water solutions for up to 30 days at room temperature (23°C) or at 4°C.[3] However, previous studies of oral formulations, which often do not specify the conditions of solution storage,[9,10] have produced conflicting results. Bhatt-Mehta et al[6] reported that 1 and 2 mg/ml of midazolam in a flavored-gelatin formulation is stable for 14 days at 4°C, although Steedman et al[8] reported that a 2.5 mg/ml flavored dye-free solution of midazolam retained more than 90% of the original concentration for 56 days when stored at temperatures up to 40°C in a flavored, dye-free syrup. However. Soy et al[10] evaluated the stability of a sweetened, flavored, and dye-free 1 mg/ml midazolam solution over 73 days using a non-specific spectrophotometric method and appear to report that only 89% remained on day 73. Yet they also report that no loss of midazolam could be detected. Gregory et al[9] reported that approximately 25% of the midazolam concentration was lost after 38 days of storage of 2.5 mg/ml midazolam in a sweetened and flavored solution. These differences do not appear to be dependent on the midazolam concentration or the presence of dye, flavoring, or sweetening agents. In fact our results confirm this; we observed less than 5% loss in the midazolam concentration over a 102-day period in a flavored, dyed, and sweetened solution up to 1 mg/ml stored at room temperature.

In conclusion, this study demonstrated that these formulations of midazolam are stable at room temperature for a period of 102 days and would be suitable for clinical use. The concentrations assessed in this study cover the range that is commonly used in clinical practice. Therefore, the clinician can be confident that no significant degradation will occur using this vehicle and that the dose assumed to be administered will be accurate.

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