Stability and Compatibility of Reconstituted Sterile Hydromorphone with Midazolam

Scott E. Walker, Antoinette Meinders and Hitesh Tailor

ABSTRACT

The stability and compatibility of combinations of hydromorphone hydrochloride (sterile Dilaudid® powder) and midazolam (Versed®) diluted in either 0.9% sodium chloride (NS) or 5% dextrose in water (D5W) was tested at 4°C and 23°C. In addition to visual inspection and pH, the concentrations of hydromorphone and midazolam in the mixtures were determined by a stability-indicating liquid chromatographic method. Within and between days analytical error, determined on replicate sample analysis, averaged less than 5% for both drugs.

Hydromorphone and midazolam were physically compatible in all tested concentration combinations when stored at 4°C or 23°C in NS and D5W solutions. The stability of four compatible combinations of hydromorphone and midazolam of: 20 mg/mL with 0.1 mg/mL; 2 mg/mL with 0.1 mg/mL; 20 mg/mL with 0.5 mg/mL; and 2 mg/mL with 0.5 mg/mL, respectively, were tested. The concentrations of both hydromorphone and midazolam in these solutions retained greater than 90% of the initial concentration for 23 days when stored at either 4°C or 23°C. The pH in these compatible solutions decreased by less than 1.0 pH unit over the study period.

In summary, we recommend a 23-day expiration date for all concentration combinations of hydromorphone and midazolam. However, expiry dates at each institution should be established giving consideration to the contamination rate within their own IV additive program.

Key Words: compatibility, hydromorphone, midazolam, stability

RÉSUMÉ

La stabilité et la compatibilité des mélanges de chlorhydrate d'hydromorphone (poudre stérile Dilaudid®) et de midazolam (Versed®) dilués dans du chlorure de sodium à 0,9 % ou du dextrose à 5 % dans l'eau ont été testées à 4 °C et à 23 °C. Outre l'inspection visuelle et la détermination du pH, on a évalué les concentrations d'hydromorphone et de midazolam dans les mélanges au moyen d'une épreuve de stabilité par chromatographie liquide. La marge d'erreur analytique pour une même journée ou entre deux journées, déterminée par une analyse d'échantillon répété, était de 5 % en moyenne pour les deux médicaments.

Les mélanges d'hydromorphone et de midazolam étaient physiquement compatibles à toutes les concentrations testées qui avaient été entreposées à 4 °C et à 23 °C dans une solution de saline normale ou de dextrose à 5% dans l'eau. La stabilité de quatre mélanges compatibles d'hydromorphone et de midazolam a été testée, soit 20 mg/mL–0,1 mg/mL, 2 mg/mL–0,1 mg/mL, 20 mg/mL–0,5 mg/mL, et 2 mg/mL–0,5 mg/mL, respectivement. Ces solutions ont retenu plus de 90 % de leurs concentrations originales d'hydromorphone et de midazolam après avoir été entreposées 23 jours à des températures de 4 °C et 23 °C. Le pH de ces mélanges de solutions compatibles a diminué de moins de 1,0 unité de pH au cours de la période d'étude.

Une durée de conservation maximale de 23 jours pour les solutions d'hydromorphone et de midazolam est donc recommandée, pour toutes les concentrations. Cependant, chaque établissement devra tenir compte du taux de contamination relatif à leur programme d'additifs aux solutés dans la détermination des durées de conservation.

Mots clés : compatibilité, hydromorphone, midazolam, stabilité.

Can J Hosp Pharm 1996;49:290-298

INTRODUCTION

Pharmacists are often asked questions regarding the compatibility of medications. Our interest in compatibility of hydromorphone with other medications stems from advances in the management of chronic pain through the development of reliable portable infusion devices.[1] The use of these devices to deliver continuous intravenous or subcutaneous infusions of narcotics to control chronic pain in cancer

Scott E. Walker, MScPhm, FCSHP, is Coordinator, Research and Quality Control, Department of Pharmacy and Division of Pharmacology, Sunnybrook Health Science Centre, North York, Ontario, and Associate Professor, Faculty of Pharmacy, University of Toronto.

Antoinette Meinders, BScN, is Medical Information Associate, Hoffmann-La Roche, Toronto, Ontario.

Hitesh Tailor, BScPharm, is Medical Information Specialist, Knoll Pharma Inc., Markham, Ontario.

Address Correspondence to: Scott Walker, Department of Pharmacy, Sunnybrook Health Science Centre, 2075 Bayview Avenue, North York, Ontario. M4N 3M5.

Acknowledgements: This study was funded jointly by Knoll Pharma Inc., and Hoffmann- La Roche Ltd. The authors would like to acknowledge the technical assistance of Alexandra Mayer and Marta Avelar.

patients has become an acceptable method of treatment.[2] In addition to improving the control of chronic pain, the use of portable infusion pumps allows patients to be managed at home.[2]

The stability of both hydromorphone[3-14] and midazolam[15-19] is well documented. However, the compatibility and stability of the combination of hydromorphone and midazolam is unknown. Therefore, it was the intent of this study to test the compatibility and stability of combinations of hydromorphone and midazolam over a 23-day period. For each combination the concentrations of hydromorphone and midazolam were evaluated by a validated stability-indicating liquid chromatographic method.

METHODS

Assay Validation

Accelerated Degradation of Hydromorphone and Midazolam.

Degradation products of both hydromorphone and midazolam were generated with acid or base and heat. A 10 mg/mL hydromorphone solution was adjusted to a pH of 8.2 using sodium hydroxide and heated at 90°C for 68 hours. The validated stability-indicating liquid chromatographic separation previously reported for hydromorphone in combination with other medications[10-14] was used to monitor separation of hydromorphone and its degradation products. Over a 68-hour period, chromatograms were inspected for the appearance of additional peaks, changes in retention time and peak shape. The UV spectral purity (200-365 nm, 6 nm bandwidth, deuterium lamp: UV3000, Thermo Separation Products, Fremont, CA) of the leading edge, middle and tail of the hydromorphone peak in a chromatogram of a degraded sample and the sample taken at time zero were also compared. The sample taken at 68 hours was retained to assist in the evaluation of the final chromatographic system.

An attempt was also made to degrade midazolam by dissolving 39.7 mg of the free base, (Lot 816072, 99.99% pure; Hoffmann-La Roche, Nutley, NJ) in 50 mL of distilled water. The pH of 10 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 and incubated in a water bath at 80°C protected from light for 50 hours. The chromatographic system for midazolam reported by Hagan et al[15] was used to monitor the formation of midazolam degradation products. 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. UV spectral purity (200-365 nm, 6 nm bandwidth, deuterium lamp: UV3000, Thermo Separation Products, Fremont, CA) of the leading edge, middle and tail of the midazolam peak in a chromatogram of a degraded sample and the sample taken at time zero were compared. The sample taken at 50 hours from the acidic aliquot (pH=2.63) was retained to assist in the evaluation of the final chromatographic system.

Chromatographic System and Separation

Following the formation of degradation products, a chromatographic separation was developed which allowed analysis of hydromorphone and midazolam simultaneously and ensured the separation of midazolam and hydromorphone from their degradation products. This chromatographic separation used a gradient based on the mobile phase of Hagan et al.[15] The initial strength of the mobile phase was much weaker than that used by Hagan et al [15] (initial percentage of organic was 40% compared to 60% used Hagan et al [15]). This mobile phase consisted of a mixture of a phosphate buffer, methanol, acetonitrile and tetrahydrofuran. The ratio of methanol: acetonitrile: tetrahydrofuran was fixed at 49:49:2. The phosphate buffer (pH 7.0) was prepared by combining 6.1 mL of 1.0 M dibasic potassium phosphate and 3.9 mL of 1.0 M monobasic potassium phosphate and diluting this mixture to 1.0 L with distilled water. The initial ratio of buffer to organic was 60:40. This percentage was held constant for three minutes and was then changed in a linear fashion over the next 14 minutes, such that by 17 minutes the ratio of buffer to organic was 28.9: 71.1. At 17 minutes the mobile phase was rapidly changed back to the initial conditions. Each sample was traced for 22 minutes. The mobile phase was pumped at 1.5 mL/min through a 25 cm x 4.2 mm C_{18}, 5um column (Ultrasphere, Beckman; Mississauga, ON) using a 600E System controller and pump (Waters Corp, Mississauga, ON). Hydromorphone and midazolam were detected at 280 nm using a scanning variable wavelength detector (UV3000; Thermo Separation Products, Freemont, CA) and chromatograms were recorded directly on computer using PC-1000 software (Thermo Separation Products, Freemont, CA). Using this separation, samples containing hydromorphone and its degradation products and midazolam and its degradation products, produced through accelerated degradation, were mixed and the UV spectral purity of hydromorphone and midazolam, relative to a fresh undegraded samples, were compared.

Assay Validation, Accuracy and Reproducibility

Validation of the method, with respect to accuracy and reproducibility was tested over a five-day period. During

this period system suitability criteria (theoretical plates, tailing and retention time) were also established for each compound of interest to ensure consistency between study days. Each sample was chromatographed in duplicate. Inter- and intra-day reproducibility were assessed using the coefficient of variation of the peak area for samples determined in duplicate and accuracy was determined based on deviations from the known concentration with both standards and quality control samples.

Compatibility Study

Physical compatibility testing was completed on the midazolam injection (Versed® - 5 mg/mL, Lot # 95116; Hoffmann-La Roche, Mississauga, ON) alone. Midazolam injection was mixed with distilled water to prepare final concentrations of 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.5, and 0.25 mg/mL. After mixing, a visual compatibility test was completed over a 24-hour period. Each solution was observed for the presence of a precipitate, colour change or evolution of gas.

The physical compatibility of a range of concentrations of hydromorphone and midazolam was also evaluated over a 24-hour period. Sterile hydromorphone hydrochloride powder (250 mg/vial, Dilaudid®, Lot # : 01080023E; Knoll Pharma Inc., Markham, ON) was reconstituted with 5 mL of sterile water to prepare a 50 mg/mL solution. Appropriate volumes of this solution were mixed with midazolam (Versed® - 5 mg/mL, Lot # 95116; - 1 mg/mL , Lot # 95182B; Hoffmann-La Roche, Mississauga, ON) to prepare 10 solutions ranging in final concentration from 0.5 to 45 mg/mL of hydromorphone and from 0.1 mg/mL to 4.5 mg/mL of midazolam. Two solutions of each concentration were prepared and the order of mixing was reversed in the second solution. After mixing, a visual compatibility test was completed over a 24-hour period. Each solution was observed for the presence of a precipitate, colour change or evolution of gas.

Stability Study

The stability and compatibility of combinations of high and low concentrations of sterile hydromorphone hydrochloride powder (250 mg/vial, Dilaudid®, Lot # : 10180023E; Knoll Pharma Inc., Markham, ON) and midazolam injection (Versed® - 5 mg/mL, Lot # 95116; Hoffmann-La Roche, Mississauga, ON) diluted in 5% dextrose in water (D5W) or 0.9% sodium chloride in water (NS), was determined. Three 10 mL aliquots of solution were prepared for each concentration-diluent-temperature combination. A total of 48 samples were prepared of four different concentration combinations, each using either D5W or NS to dilute the injectable solutions. These solutions had initial hydromorphone and midazolam concentrations following mixing of: 20 mg/mL with 0.1 mg/mL; 2 mg/mL with 0.1 mg/mL; 20 mg/mL with 0.5 mg/mL; and 2 mg/mL with 0.5 mg/mL, respectively. Equal numbers of solutions were stored at room temperature (23°C) and in the refrigerator (4°C). One container of each concentration-diluent-temperature combination was used to complete the physical inspection and pH while the remaining two containers were used to determine the concentration of hydromorphone and midazolam by liquid chromatographic analysis on days 0, 1, 2, 4, 7, 9, 16, 18, 21 and 23.

Liquid Chromatographic Analysis.

On each study day, fresh standards of hydromorphone and midazolam were chromatographed to construct a standard curve. A stock solution of midazolam was prepared by dissolving an accurately weighed quantity of approximately 20 mg of midazolam powder, as the free base, (Lot 816072, 99.99% pure; Hoffmann-La Roche, Nutley, NJ) in 10 mL of a solution of 50% methanol and 50% distilled water. This stock solution of approximately 2 mg/mL was then diluted to prepare six concentrations of 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 mg/mL. These six standards plus a blank were used to construct a standard curve. Three quality control samples were prepared from the same stock solution of midazolam with final nominal concentrations of 0.10 mg/mL, 0.25 and 0.50 mg/mL. Five microlitres of each standard, quality control sample and a blank were directly chromatographed in duplicate on each study day.

A stock solution of hydromorphone was prepared by dissolving an accurately weighed quantity of approximately 100 mg of sterile hydromorphone hydrochloride powder (Dilaudid®, Lot # L50150094; Knoll Pharma Inc., Markham, ON) in 2 mL of distilled water. This stock solution of 50 mg/mL was then diluted to prepare six concentrations of 1.0, 2.5, 5.0, 10.0, 18.75, and 25.0 mg/mL. Five microlitres of each of these six standards and a blank were directly chromatographed in duplicate and the concentration of hydromorphone determined. Three quality control samples of 2.0, 10.0, and 20.0 mg/mL were prepared from the same stock solution of hydromorphone hydrochloride.

Hydromorphone and midazolam were quantified simultaneously each day using the same reverse phase gradient liquid chromatographic separation described under Chromatographic System and Separation. The average peak area of two replicates from each sample of hydromorphone and midazolam were subjected to least squares linear regression and the concentration was interpolated from standard curves and recorded. Concentrations were recorded to the nearest 0.001 mg/mL.

pH and Physical Inspection

Physical inspection was completed on solutions as they were drawn for chemical analysis. On each of the study days a 1 mL sample was drawn and placed in a 10 x 75 mm glass test tube. Each solution was inspected visually for colour and clarity. The pH of each solution was then measured. The pH meter (Accumet-model 925; Fisher Scientific, Toronto, ON) was equipped with a microprobe glass body electrode (cat# 13-639-280; Fisher Scientific, Toronto, ON) and was standardized each day with two commercially available buffer solutions. The pH was recorded to the nearest 0.001 of a pH unit.

Data Reduction and Statistical Analysis

Means (± standard deviation) were calculated for replicated analyses. Reproducibility was assessed by coefficient of variation (CV). Mean concentration results for each solution were analysed by least squares linear regression to determine the percent of initial concentration remaining on the last day of the study. Multiple linear regression and analysis of variance (SPSS for Windows®, Release 5.0.1, 1992) were used to compare differences between temperatures, diluents and concentrations for similar analytical tests. The five percent level was used as the *a priori* cut-off for significance.

Hydromorphone and midazolam concentrations were considered "within acceptable limits" if the concentration on any day of analysis was not less than 90% of the initial (day-zero) concentration. A solution was judged to be physically compatible if there was no visual change in the colour or clarity of the mixture and no precipitate or other particulate formation was visually apparent.

RESULTS

Assay Validation

Accelerated Degradation of Hydromorphone

At the end of the 68-hour accelerated degradation study period approximately 70% of the initial hydromorphone concentration remained and there was chromatographic evidence of a degradation product in the solvent front. This degradation product did not interfere with hydromorphone quantification with either the initial isocratic or final gradient separations. The UV spectral purity of the hydromorphone peak remained identical to an authentic hydromorphone standard. The predictable degradation, chromatographic separation of hydromorphone from midazolam and the degradation products of both compounds (Figure 1), and the UV spectral homogeneity of a degraded sample, demonstrated that this analytical method was stability-indicating for hydromorphone.[20, 21]

Accelerated Degradation of Midazolam

At a pH of 2.63, the sample drawn prior to incubation was observed to have a large peak, which eluted prior to midazolam and could be separated completely from midazolam by both the isocratic mobile phase used to monitor degradation and the gradient used in the stability study (Figure 1). However, the size of this peak did not change over the 50-hour study period and at all other pHs less than 10% degradation occurred. Nevertheless, the UV spectral purity of the midazolam peak in the sample from pH 2.63 remained identical to an authentic midazolam standard. The ability of the chromatographic gradient system to completely separate midazolam from this other compound (a ring opened benzophenone[15, 22, 23]), chromatographic separation of midazolam from hydromorphone and the degradation products of hydromorphone (Figure 1), and the UV spectral homogeneity of a degraded sample, demonstrated that this analytical method was stability-indicating for midazolam.[20, 21]

Assay Validation, Hydromorphone

Duplicate analysis of hydromorphone quality control samples (concentrations of 20, 10 and 2 mg/mL), demonstrated that concentrations were estimated with less than a 6% deviation between the observed and known concentration and the variation (CV%) on duplicate analysis was approximately 1%, within a day, and

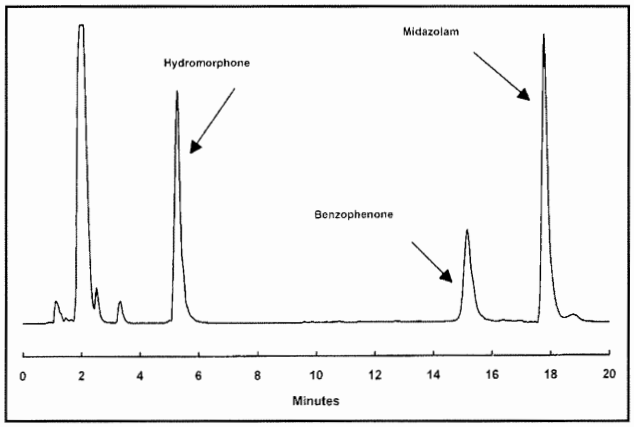

Figure 1: This chromatogram demonstrates the ability of the chromatographic gradient system to completely separate midazolam and hydromorphone from the ring opened benzophenone, hydromorphone degradation products and other sample contaminants. The UV spectral homogeneity of midazolam and hydromorphone in this sample and their similarity to authentic standards demonstrated that this analytical method was stability-indicating for midazolam and hydromorphone.

less than 4.3% between days. Accuracy and reproducibility for standards was similar. Deviations from the known concentration were routinely within 3% and error (CV%) of duplicate analysis, within a day, ranged from 0.02% to 5.92%, and averaged less than 2% for all concentrations. These analyses indicated that the hydromorphone concentrations were measured accurately and reproducibly and that differences of 10% or more could be confidently detected with acceptable error rates.[24, 25]

Assay Validation, Midazolam

The accuracy of midazolam, based on duplicate analysis of quality control samples (concentrations of 0.5 , 0.25 and 0.1 mg/mL), demonstrated that concentrations were estimated with deviations of less than 6% and the error (CV%) on duplicate analysis, was approximately 1.6% within a day, and was less than 3.83% between days. Accuracy and reproducibility for standards was similar. Deviations from known concentration were routinely within 5% and error (CV%) of duplicate analysis, within a day, ranged from 0.09% to 5.52%, averaging approximately 2% for all concentrations. These analyses indicated that the midazolam concentrations were measured accurately and reproducibly and that differences of 10% or more could be confidently detected with acceptable error rates.[24, 25]

Compatibility/Stability Studies

At room temperature over a 24-hour period, solutions of hydromorphone (0.5 to 45 mg/mL) and midazolam (0.1 to 4.5 mg/mL) were observed to be physically compatible. No precipitate was visible in any solution, no colour changes occurred and no gas was produced on mixing. Furthermore, solutions of midazolam injection (0.25 to 4 mg/mL), diluted with distilled water, were also observed to be physically compatible over the 24-hour study period.

During the 23-day stability study period, neither hydromorphone nor midazolam degraded to a measurable extent. The hydromorphone concentration remaining on the last study day in all samples was greater than 93.64% of the initial concentration (range 93.64% to 99.53% - Tables I and II). For each concentration-solution-temperature combination the fluctuation in concentration was similar to assay error, averaging 2.26% (range: 1.23% to 3.22%). These changes in concentration were not significant and so the variables of temperature (4°C and 23°C; p = 0.9926), diluent (NS or D5W; p = 0.9160) and midazolam concentration (0.1 and 0.5 mg/mL; p = 0.9513), did not significantly affect the stability of hydromorphone over the duration of this study period.

In all samples the midazolam concentration remaining on the last study day was greater than 93.07% of the

Table I: Observed Percent Remaining of Hydromorphone in Normal Saline Solutions

Day	4 °C				23°C			
	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]
0	100.00 ± 0.55	100.00± 0.48	100.00 ± 0.48	100.00 ± 0.35	100.00 ± 1.12	100.00 ± 1.34	100.00 ± 0.05	100.00 ± 0.31
1	100.25 ± 0.32	108.86± 3.79	98.65 ± 0.37	98.94 ± 0.03	100.72 ± 0.14	107.27 ± 1.45	100.43 ± 0.16	100.45 ± 0.95
2	101.77 ± 0.03	103.84± 0.40	100.15 ± 0.37	100.09 ± 1.60	101.69 ± 0.38	104.24 ± 0.71	101.14 ± 0. 08	101.5 ± 1.11
4	100.92 ± 0.19	102.18± 2.59	99.80 ± 0.30	103.27 ± 0.94	100.61 ± 0.17	100.91 ± 0.82	100.56 ± 1.08	99 .85 ± 1.40
7	100.50 ± 0.48	102.97± 0.92	100.51 ± 0.19	102.42 ± 0.40	100.40 ± 0.42	103.39 ± 1.23	100.09 ± 0.20	106.69 ± 8.17
9	100.08 ± 0.30	101.18± 0.22	98.11 ± 0.31	98.68 ± 0.51	99.71 ± 0.08	102.87 ± 0.72	99.42 ± 0.29	99.15 ± 0.03
16	95.07 ± 1.88	106.83± 1.78	95.27 ± 5.22	98.14 ± 1.48	100.36 ± 1.93	105.70 ± 1.55	99.66 ± 0.30	100.94 ± 1.28
18	93.18 ± 1.13	101.95± 5.48	96.58 ± 2.55	98.68 ± 1.17	98.98 ± 0.61	105.26 ± 1.76	99.78 ± 0.97	105 52 ± 0.40
21	98.74 ± 8.63	103.10 ± 6.08	98.29 ± 7.62	96.58 ± 2.28	97.92 ± 0.14	99.88 ± 0.26	99.14 ± 6.33	101.55 ± 0.21
23	94.56 ± 0.40	100.14 ± 3.07	97.64 ± 4.99	96.15 ± 2.27	96.51 ± 2.61	96.11 ± 1.16	91.57 ± 3.58	96.40 ± 6.19
CV(%)[5]	3.01	2.72	1.70	2.24	1.50	3.22	2.70	2.94
Percent Remaining on Day 23[6]	93.64	98.56	96.92	95.81	96.71	96.88	95.39	99.47

1. H20 represents hydromorphone (20 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial hydromorphone concentrations of 20.67 and 20.61 mg/mL, respectively.
2. H2 represents hydromorphone (2 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial hydromorphone concentrations of 2.08 and 2.08 mg/mL, respectively.
3. H20 represents hydromorphone (20 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial hydromorphone concentrations of 20.85 and 20.65 mg/mL, respectively.
4. H2 represents hydromorphone (2 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial hydromorphone concentrations of 2.10 and 2.10 mg/mL, respectively.
5. Variability of estimated concentrations over the study period expressed as coefficient of variation.
6. Percent Remaining (%) on day 23 based on linear regression. Concentrations on day 23 and day zero determined by linear regression. Calculation : [Day 23 *100 / Day zero].

Table II: Observed Percent Remaining of Hydromorphone in 5% Dextrose in Water Solutions

Day	4 °C				23°C			
	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]
0	100.00 ± 0.13	100.00 ± 0.00	100.00 ± 0.57	100.00 ± 1.55	100.00 ± 0.50	100.00 ± 0.05	100.00 ± 0.58	100.00 ± 1.73
1	100.69 ± 0.13	99.41 ± 0.62	98.76 ± 0.10	99.06 ± 4.20	99.22 ± 0.52	98.51 ± 0.18	99.91 ± 1.32	96.20 ± 0.10
2	100.30 ± 0.37	101.91 ± 0.57	98.68 ± 0.44	98.00 ± 1.61	100.33 ± 0.55	100.71 ± 1.06	104.07 ± 3.73	97.60 ± 0.67
4	101.25 ± 0.85	99.43 ± 0.90	101.52 ± 0.55	100.19 ± 0.23	100.58 ± 0.70	103.05 ± 0.40	99.60 ± 3.01	98.72 ± 3.48
7	100.18 ± 1.27	101.50 ± 1.23	99.24 ± 0.11	101.15 ± 0.62	99.91 ± 0.31	99.55 ± 0.35	100.14 ± 1.93	99.64 ± 1.46
9	99.47 ± 0.05	99.35 ± 1.01	97.87 ± 0.20	100.43 ± 0.32	100.13 ± 1.61	97.86 ± 0.44	99.34 ± 0.67	100.71 ± 2.23
16	99.12 ± 0.39	100.67 ± 4.39	97.65 ± 0.64	98.35 ± 2.54	97.90 ± 0.53	98.93 ± 3.49	101.90 ± 0.91	101.38 ± 0.70
18	98.02 ± 0.96	101.67 ± 0.45	97.96 ± 1.52	96.53 ± 0.87	97.71 ± 1:47	98.46 ± 2.54	98.09 ± 0.97	98.51 ± 1.19
21	97.27 ± 1.34	98.02 ± 8.11	98.37 ± 2.71	99.34 ± 0.12	96.17 ± 0.85	104.29 ± 3.76	94.29 ± 4.52	96.49 ± 4.03
23	97.85 ± 0.58	100.07 ± 5.45	97.27 ± 0.81	93.35 ± 4.02	95.87 ± 0.05	94.74 ± 2.06	93.95 ± 3.28	92.55 ± 1.67
CV(%)[5]	1.31	1.23	1.27	2.29	1.74	2.68	3.04	2.67
Percent Remaining on Day 23[6]	96.83	99.53	97.78	96.28	95.82	98.43	94.23	97.42

1. H20 represents hydromorphone (20 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial hydromorphone concentrations of 20.79 and 20.80 mg/mL, respectively.
2. H2 represents hydromorphone (2 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial hydromorphone concentrations of 2.11 and 2.16 mg/mL, respectively.
3. H20 represents hydromorphone (20 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL). with initial hydromorphone concentrations of 21.09 and 21.14 mg/mL, respectively.
4. H2 represents hydromorphone (2 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial hydromorphone concentrations of 2.19 and 2.26 mg/mL, respectively.
5. Variability of estimated concentrations over the study period expressed as coefficient of variation.
6. Percent Remaining (%) on day 23 based on linear regression. Concentrations on day 23 and day zero determined by linear regression. Calculation : [Day 23 * 100 / Day zero].

initial concentration (range 93.07% to 102.23 - Tables III and IV). For each concentration-solution-temperature combination the fluctuation in concentration was similar to assay error, averaging 2.92% (range: 1.75% to 4.27%). These changes in concentration were not significant and so the variables of temperature (4°C and 23°C; p = 0.9397), diluent (NS and D5W; p = 0.9795) and hydromorphone concentration (2 and 20 mg/mL; p=0.9883), did not significantly affect midazolam stability over the study period.

pH and Physical Inspection

The pH of a 50 mg/mL hydromorphone hydrochloride (Dilaudid® sterile powder) solution in water was 4.61 and the pH of a 5 mg/mL midazolam hydrochloride (Versed®) solution in water was 3.73. The pH of solutions appeared to be primarily dependant on the midazolam concentration. Solutions containing 0.5 mg/mL of midazolam had a mean initial pH of 3.66 ± 0.12, ranging from 3.63 to 3.84, whereas solutions containing 0.1 mg/mL of midazolam had a mean initial pH of 5.15 ± 0.44, ranging from 4.98 to 5.95. During the 23-day study period there was a small, but consistent and significant ($p < 0.005$) reduction in pH from the initial pH of 4.62 ± 0.94 to 4.19 ± 0.64 on day 23. In most samples (13 of 16), the reduction in pH was less than 0.80 of a pH unit (range -0.05 to -0.79), although in three samples (all containing 0.1 mg/mL of midazolam), the pH dropped by 1.3 units over the 23-day study period.

DISCUSSION

Least squares linear regression of the change in concentration with time demonstrated that there was less than a 7% change in concentration for both midazolam and hydromorphone over the 23-day study period. In studies where no change in the concentration of the drugs of interest can be detected, assurance that the analytical method is specific for the compound of interest is important. This was demonstrated in the accelerated degradation portion of the study where we were able to separate degradation products from both hydromorphone and midazolam. In the case of midazolam, true degradation did not occur as degradation beyond the appearance of an additional peak at time zero in acidic solution did not continue during the 50-hour study period. It is known that at a pH of less than 3.3, a ring opened structure [benzophenone] is formed.[15,22,23] This compound is in equilibrium with midazolam and is favoured over the ring closed structure of midazolam in acidic media.[15,22,23] Since it can revert completely to midazolam at a pH of 7.4[15,22,23] or greater, this compound is not a true degradation product. It has been previously reported that the ring opened structure cannot be

Table III: Observed Percent Remaining of Midazolam in Normal Saline Solutions

Day	4 °C				23°C			
	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]
0	100.00 ± 0.44	100.00 ± 0.47	100.00 ± 1.89	100.00 ± 0.17	100.00 ± 0.63	100.00 ± 1.93	100.00 ± 0.52	100.00 ± 0.24
1	98.19 ± 0.11	97.23 ± 1.42	98.56 ± 0.61	102.43 ± 0.40	97.48 ± 1.12	103.25 ± 0.24	100.42 ± 0.32	102.51 ± 0.04
2	98.06 ± 0.30	97.79 ± 0.95	98.26 ± 2.10	97.23 ± 0.44	101.22 ± 1.34	97.30 ± 2.01	98.90 ± 0.57	99.62 ± 2.20
4	102.53 ± 0.38	100.22 ± 1.29	97.61 ± 0.03	101.34 ± 0.86	99.56 ± 2.23	99.34 ± 3.75	98.17 ± 0.26	101.03 ± 0.59
7	96.05 ± 0.66	100.82 ± 1.60	97.07 ± 0.30	99.62 ± 0.74	96.97 ± 2.00	97.76 ± 0.79	94.84 ± 0.24	99.34 ± 0.44
9	95.94 ± 0.73	92.10 ± 0.25	95.90 ± 0.50	97.67 ± 1.22	95.32 ± 0.21	94.21 ± 0.61	98.97 ± 1.10	99.36 ± 2.96
16	94.02 ± 1.32	92.65 ± 2.33	97.30 ± 4.66	95.96 ± 0.60	96.44 ± 0.33	96.91 ± 1.54	97.95 ± 0.96	98.84 ± 1.18
18	98.63 ± 1.39	93.88 ± 0.22	95.34 ± 4.93	93.51 ± 1.86	104.11 ± 4.70	100.29 ± 6.40	98.91 ± 1.85	96.78 ± 1.62
21	98.74 ± 1.58	93.62 ± 2.89	94.14 ± 1.02	95.25 ± 1.78	94.48 ± 0.36	95.68 ± 1.45	102.91 ± 2.67	100.01 ± 1.59
23	102.88 ± 3.40	102.59 ± 7.92	95.86 ± 3.38	94.12 ± 2.04	105.16 ± 3.87	101.23 ± 0.72	97.08 ± 0.66	94.83 ± 4.37
CV(%)[5]	2.85	3.87	1.75	3.04	3.67	2.75	2.16	2.11
Percent Remaining on Day 23[6]	100.37	97.26	96.07	93.07	101.65	98.73	102.23	95.92

1. H20 represents hydromorphone (20 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial midazolam concentrations 0.103 and 0.104 mg/mL, respectively.
2. H2 represents hydromorphone (2 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial midazolam concentrations 0.107 and 0.105 mg/mL, respectively.
3. H20 represents hydromorphone (20 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial midazolam concentrations 0.507 and 0.504 mg/mL, respectively.
4. H2 represents hydromorphone (2 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial midazolam concentrations 0.511 and 0.510 mg/mL, respectively.
5. Variability of estimated concentrations over the study period expressed as coefficient of variation.
6. Percent Remaining (%) on day 23 based on linear regression. Concentrations on day 23 and day zero determined by linear regression. Calculation : [Day 23 ± 100 / Day zero].

Table IV. Observed Percent Remaining of Midazolam in 5% Dextrose in Water Solutions

Day	4 °C				23°C			
	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]	H20:0.1M[1]	H2:0.1M[2]	H20:0.5M[3]	H2:0.5M[4]
0	100.00 ± 2.84	100.00 ± 2.05	100.00 ± 0.26	100.00 ± 0.64	100.00 ± 0.35	100.00 ± 0.61	100.00 ± 0.77	100.00 ± 1.78
1	100.48 ± 1.35	100.10 ± 0.68	101.44 ± 1.29	101.36 ± 1.62	96.07 ± 1.40	98.69 ± 1.71	101.82 ± 2.09	99.77 ± 0.32
2	97.16 ± 1.77	100.87 ± 0.13	96.24 ± 0.42	96.61 ± 0.33	107.22 ± 1.80	100.66 ± 1.33	101.95 ± 2.10	97.50 ± 0.64
4	100.09 ± 0.68	101.60 ± 0.84	98.65 ± 0.66	98.49 ± 1.85	104.58 ± 1.38	98.34 ± 0.18	98.21 ± 3.84	101.05 ± 1.41
7	95.64 ± 2.08	97.80 ± 4.23	95.79 ± 0.04	97.53 ± 0.32	99.74 ± 4.53	96.40 ± 2.25	96.25 ± 0.14	97.12 ± 2.22
9	95.39 ± 0.11	93.91 ± 0.49	95.73 ± 1.78	97.55 ± 2.68	95.17 ± 1.03	94.05 ± 2.48	96.95 ± 0.53	93.18 ± 1.94
16	92.40 ± 1.16	99.32 ± 1.11	99.95 ± 1.47	93.92 ± 2.00	97.53 ± 0.88	95.32 ± 4.20	100.29 ± 1.52	95.12 ± 1.45
18	99.16 ± 1.83	104.62 ± 3.02	97.61 ± 0.77	94.85 ± 1.02	98.45 ± 3.65	103.74 ± 0.36	97.87 ± 0.34	98.14 ± 3.45
21	96.61 ± 1.80	100.35 ± 6.02	97.86 ± 2.08	97.08 ± 2.07	99.68 ± 6.08	97.19 ± 4.95	95.57 ± 4.02	95.55 ± 1.39
23	103.96 ± 2.23	102.03 ± 0.49	96.74 ± 0.12	93.09 ± 3.52	106.90 ± 0.91	103.91 ± 6.12	92.15 ± 2.72	91.95 ± 0.34
CV(%)[5]	3.36	2.84	2.00	2.60	4.27	3.38	3.03	3.01
Percent Remaining on Day 23[6]	99.98	101.99	98.59	94.47	100.48	101.93	94.26	94.44

1. H20 represents hydromorphone (20 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial midazolam concentrations 0.107 and 0.103 mg/mL, respectively.
2. H2 represents hydromorphone (2 mg/mL) and 0.1M indicates midazolam (0.1 mg/mL) with initial midazolam concentrations 0.104 and 0.106 mg/mL, respectively.
3. H20 represents hydromorphone (20 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial midazolam concentrations 0.513 and 0.512 mg/mL, respectively.
4. H2 represents hydromorphone (2 mg/mL) and 0.5M indicates midazolam (0.5 mg/mL) with initial midazolam concentrations 0.508 and 0.514 mg/mL, respectively.
5. Variability of estimated concentrations over the study period expressed as coefficient of variation.
6. Percent Remaining (%) on day 23 based on linear regression. Concentrations on day 23 and day zero determined by linear regression. Calculation : [Day 23 * 100 / Day zero].

completely separated from midazolam,[15,22,23] however, the gradient used in this study was able to completely separate these compounds. Hagan et al[15] reported that an additional degradation product appeared in the solvent front after storage in acid for one hour at room temperature. In our study at 80°C, one additional peak was also observed to elute in the solvent front of chromatograms of acidic samples. However, the size of this peak did not change over the study period and our conditions were more extreme than those reported by Hagan et al.[15] Nevertheless, the ability to separate the ring opened benzophenone and hydromorphone degradation products from both midazolam and hydromorphone and the UV spectral purity of midazolam and hydromorphone indicated to that this method was specific for the compounds of interest and was, therefore, stability-indicating.

A number of reports have been published concerning hydromorphone compatibility with various drugs.[4-14] Physical incompatibilities are often concentration dependant, such that lower concentrations of one or both of the medications may change an incompatible mixture to a compatible solution. Physical incompatibilities with hydromorphone have been observed with minocycline,[9] tetracycline,[9] dexamethasone,[10] phenytoin,[11] phenobarbital,[11] diazepam,[11] cloxacillin in D5W,[11] high concentrations of cefazolin,[9,11] dimenhydrinate,[13] and heparin.[14] Hydromorphone has also been observed to inactivate hyaluronidase and so while the combination was judged to be physically compatible, it is chemically unstable.[12] A similar observation has been made with the combination of hydromorphone and lorazepam where the stability of lorazepam in the combination limits the expiry date which can be placed on the combination.[13] While physical compatibility between hydromorphone and a variety of medications has been reported,[6-9] these studies only assessed compatibility visually over no more than four hours. The demonstration of chemical compatibility-stability of hydromorphone over a period of 24 hours or more, through liquid chromatographic assay, has been reported for mixtures of ampicllin,[11] ceftazidime,[11] prochlorperazine,[13] potassium chloride[14] with hydromorphone. This current study has also demonstrated chemical compatibility over 23 days between midazolam and hydromorphone.

Midazolam is also a very stable compound. More than 90% of the initial concentration is retained in NS or D5W solutions for up to 30 days at room temperature (23°C) or at 4°C,[15] in parenteral nutrition solutions for five hours,[16] in flavoured gelatin for 28 days [17] and polypropylene syringes for 13 days.[18] Midazolam has also been shown to be chemically compatible and stable in combination with morphine[19] over a 14-day period. This current study has also demonstrated chemical compatibility over 23 days between midazolam and hydromorphone.

In this study, the only discordant result was a decrease in the pH by approximately 1.3 pH units in three vials while the reduction in pH in the remaining 13 averaged less than 0.25 pH units. The three solutions in which the large reduction in pH occurred all contained 0.1 mg/mL of midazolam and 2 mg/mL of hydromorphone. This change in pH did not affect the stability of either drug and the cause of the change in pH is not immediately apparent. However, since these solutions are not maintained in a sterile environment during the study, it is possible that the reduction in pH was caused by microbial contamination.

In this study, midazolam and hydromorphone were found to be physically compatible and chemically stable with each other in all concentration, temperature, and diluent combinations. Therefore, we recommend a 23-day expiration date for all concentration combinations of hydromorphone and midazolam when these solutions are stored in NS or D5W at either 4°C or 23°C. These solutions will retain more than 90% of the original hydromorphone and midazolam concentrations over this 23-day period. However, expiry dates at each institution should be established giving consideration to the contamination rate within their own IV additive program.

REFERENCES

1. Coons CE, Sone M. Portable narcotic infusion devices in Canada. *Can J Hosp Pharm* 1989; 42: 235-8.
2. Swanson G, Smith J, Bulich R, New P. Shiffman R. Patient-controlled analgesia for chronic cancer pain in the ambulatory setting: A report of 117 patients. *J Clin Oncol* 1989; 7: 1903-8.
3. Walker SE, Coons C, Matte D, De Angelis C, Iazzetta J, Kolos C, et al. Stability of hydromorphone solutions and morphine solutions in portable infusion pump cassettes and minibags. *Can J Hosp Pharm* 1988; 41: 177-82.
4. Ingallinera T, Kapadia AJ, Hagman D, Klioze O. Compatibility of glycopyrrolate injection with commonly used infusion solutions and additives. *Am J Hosp Pharm* 1979; 36: 508-10.
5. Anon. Vistril IM, Table of physical compatibilities. Pfizer Laboratories. New York, New York. July 1979.
6. Cutie MR. Small volume parenterals mixed with dilaudid hydrochloride ampuls and vials. *Hosp Formul* 1980; 15: 502-3.
7. Souney PF, Solomon MA, Stancher D. Visual compatibility of cimetidine with common pre-operative injectable medications. *Am J Hosp Pharm* 1984; 41:1840-1
8. Parker WA. Physical compatibility of ranitidine HCl with preoperative injectable medications. *Can J Hosp Pharm* 1985; 38: 160-1.
9. Nieves-Cordero AL, Luciw HM, Souney PF. Compatibility of narcotic analgesic solutions with various antibiotics during simulated Y-site injection. *Am J Hosp Pharm* 1985; 42: 1108-9.
10. Walker SE, De Angelis C, Iazzetta J, Eppel JG. Compatibility of dexamethasone sodium phosphate with hydromorphone

hydrochloride or diphenhydramine hydrochloride. *Am J Hosp Pharm* 1990; 48: 2161-6.
11. Walker SE, De Angelis C, Iazzetta J. Stability and compatibility of combinations of hydromorphone and a second drug. *Can J Hosp Pharm* 1991: 44; 289-95.
12. Walker SE, Lau DWC. Compatibility and stability of hyaluronidase and hydromorphone. *Can J Hosp Pharm* 1992; 45: 187-92.
13. Walker SE, Iazzetta J, De Angelis C, Lau DWC. Stability and compatibility of combinations of hydromorphone and dimenhydrinate, lorazepam, or prochlorperazine. *Can J Hosp Pharm* 1993; 46: 61-5.
14. Avelar M, Walker SE. Compatibility and stability of hydromorphone with heparin or KCl. *Can J Hosp Pharm* 1995; 49: 140-5.
15. Hagan RL, Jacobs LF, Pimsler M, Merritt GJ. Stability of midazolam hydrochloride in 5% dextrose injection or 0.9% sodium chloride injection over 30 days. *Am J Hosp Pharm* 1993; 50: 2379-81.
16. Bhatt-Mehta V, Rosen DA, King RS, Maksym CJ. Stability of midazolam hydrochloride in parenteral nutrition solutions. *Am J Hosp Pharm* 1993; 50: 285-8.
17. Bhatt-Mehta V, Johnson CE, Kostoff L, Rosen DA. Stability of midazolam hydrochloride in extemporaneously prepared flavored gelatin. *Am J Hosp Pharm* 1993; 50: 472-5.
18. Peterson GM, Khoo BH, Galloway JG, Paterson J. Preliminary study of the stability of midazolam in polypropylene syringes. *Aust J Hosp Pharm* 1991; 21: 115-8.
19. LeBelle MJ, Savard C, Gagnon A. Compatibility of morphine and midazolam or haloperidol in parenteral admixtures. *Can J Hosp Pharm* 1995; 48: 155-60.
20. Trissel LA. Avoiding common flaws in stability and compatibility studies of injectable drugs. *Am J Hosp Pharm* 1983; 40:1159-60
21. Trissel LA, Flora KP. Stability studies: five years later. *Am J Hosp Pharm* 1988; 45:1569-71.
22. Andersin R. Solubility and acid-base behaviour of midazolam in media of different pH, studied by ultraviolet spectrophotometry with multicomponent software. *J Pharm Biomed Anal* 1991; 9: 451-5.
23. Han WW, Yakatan GJ, Maness DD. Kinetics and mechanism of hydrolysis of 1,4 benzodiazepines. I: chlordiazepoxide and demoxepam. *J Pharm Sci* 1976; 65: 1198-204.
24. Frieman JA, Chalmers TC, Smith H, Kuebler RR. The importance of beta, Type II error and sample size in the design and interpretation of the randomized control trial. *N Engl J Med* 1978; 299:690-4.
25. Stolley PD, Strom BL. Sample size calculations for clinical pharmacology studies. *Clin Pharmacol Therap* 1986; 39:489-90.