Stability of Dolasetron Mesylate in 0.9% Sodium Chloride and 5% Dextrose in Water

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ABSTRACT

Objective: To reduce the cost of the IV additive program at the institution of one of the authors, the Pharmacy Department began to dispense IV medications in syringes. To facilitate this program it was of interest to determine the stability of a dolasetron myselate solution that was half the concentration (10 mg/mL) of the commercially available product (Anzemet, Hoechst Marion Roussel, Montreal, Quebec); the more dilute solution was to be repackaged and stored in 3-mL polypropylene syringes.

Methods: On study day zero, dolasetron mesylate for injection 20 mg/mL was diluted with an equal volume of either 0.9% sodium chloride (normal saline; NS) or 5% dextrose in water (D5W). Aliquots were drawn into 3-mL polypropylene syringes, which were stored in a refrigerator (at 4°C) or at room temperature (23°C). On study days 0, 2, 3, 6, 8, 10, 14, 16, 21, 23, 29, and 31, the concentration of dolasetron was determined (by means of a validated reverse-phase stability-indicating liquid chromatographic method with ultraviolet detection at 230 nm), a physical inspection was completed, and the pH was measured.

Results: During the study period, all samples retained at least 96.1% of the initial dolasetron concentration. There was no significant effect of time, temperature, or solvent on dolasetron stability. No degradation products were revealed by inspection of chromatograms obtained during the stability study. All solutions were initially clear and colourless and remained so for the duration of the study. There was no significant change in pH over the study period.

Conclusions: Solutions of dolasetron mesylate 10 mg/mL prepared in NS or D5W and stored in polypropylene syringes at 4°C or 23°C retained more than 96% of their initial concentration during 31 days of storage.

Key words: dolasetron, stability

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RÉSUMÉ

Objectif : Afin de réduire le coût du programme d'additifs aux solutés de l'établissement auquel un des auteurs est rattaché, le département de pharmacie a commencé à distribuer des médicaments intraveineux dans des seringues. Pour faciliter ce programme, on a jugé pertinent de déterminer la stabilité des solutions de mésylate de dolasétron préparées à une concentration deux fois moindre (10 mg/mL) que celle du produit disponible dans le commerce (Anzemet, Hoechst Marion Roussel Canada Inc., Montréal (Québec)); les solutions ainsi diluées étaient reconditionnées dans des seringues de 3 mL en polypropylène.

Méthodes: Au jour 0 de l'étude, le dolasétron pour injection à 20 mg/mL a été dilué dans un volume égal de chlorure de sodium à 0,9 % (NS) ou de solution aqueuse de dextrose à 5 % (D5W) ; les aliquots ont été aspirés dans les seringues de 3 mL en polypropylène, qui ont été conservées dans un réfrigérateur (à 4 °C) ou entreposées à la température ambiante (23 °C). Aux jours 0, 2, 3, 6, 8, 10, 14, 16, 21, 23, 29 et 31, on a déterminé la concentration de dolasétron (au moyen d'une épreuve de stabilité par chromatographie liquide en phase inverse avec détection UV à 230 nm), effectué une inspection visuelle et mesuré le pH des solutions.

Résultats : Au cours de l'étude, tous les échantillons ont conservé au moins 96,1 % de leur concentration initiale de dolasétron. Aucun effet significatif sur la stabilité du dolasétron lié au temps, à la température ou au diluant n'a été observé. L'inspection des chromatogrammes n'ont révélé la présence d'aucun produit de dégradation. Toutes les solutions étaient au départ limpides et incolores et le sont demeurées pendant toute la durée de l'étude. Aucun changement significatif du pH n'a été observé au cours de l'étude.

Conclusions : Les solutions de mésylate de dolasétron à des concentrations de 10 mg/mL de NS ou de D5W, entreposées dans des seringues de polypropylène à 4 °C ou à 23 °C ont conservé plus de 96 % de leur concentration initiale de dolasétron pendant 31 jours.

Mots clés : dolasétron, stabilité

INTRODUCTION

In 1997, to reduce the cost of the IV additive program, the Pharmacy Department at the Thunder Bay Regional Hospital began to dispense IV medications in syringes. The list of approved drugs that nurses may administer by direct IV administration includes dolasetron. The maximal dose of dolasetron used in postoperative nausea and vomiting is 12.5 mg, far lower than that used for chemotherapy- and radiation-induced nausea and vomiting (100 mg).[1-3] However, dolasetron is currently available only in a 5-mL vial containing a 20 mg/mL solution.

The product monograph for this formulation, Anzemet, indicates that solutions of 100 mg in 50 mL of 0.9% sodium chloride (normal saline; NS), 5% dextrose in water (D5W), or other common IV solutions should be used immediately or stored between 2°C and 8°C for no more than 24 h.[4] However, all previous reports of dolasetron stability[4,5] dealt with solutions of 1 or 2 mg/mL and hence were not applicable to the concentration of interest (10 mg/mL). Therefore, the purpose of this study was to determine the stability of a 10 mg/mL solution of dolasetron, prepared by dilution of the commercial product with NS or D5W and then drawn into 3-mL polypropylene syringes.

METHODS

Assay Validation

Two of the authors had previously developed and validated a stability-indicating assay for dolasetron in combination with dexamethasone.[5] Before initiation of the study reported here, this method was revalidated to ensure continued accuracy and reproducibility of the method and to confirm that dolasetron could be separated from its degradation products.

After the development of the chromatographic system for dolasetron, the suitability of this method for use as a stability-indicating assay[6,7] was tested by analyzing samples generated by accelerated degradation of dolasetron. One millilitre of a 20 mg/mL solution of dolasetron mesylate for injection (Anzemet, Hoechst Marion Roussel Canada Inc, Montreal, Quebec, lot A0D24, expiry October 2002) was diluted with 9 mL of distilled water to make a 2 mg/mL stock solution. To accelerate the degradation of dolasetron, 0.02 mL of 1% sodium hypochlorite (Hygeol, a stabilized sodium hypochlorite solution equivalent to 1% available chlorine; Wampole Canada Inc, lot 0B030A, expiry February 2002) was added to 1 mL of this stock solution. A sample of the solution was taken for chromatography immediately after the addition of the sodium hypochlorite solution, and other samples were taken after 13, 30, 48, 72, 110 and 151 min. The chromatograms of these samples were inspected for the appearance of additional peaks, and the dolasetron 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 study day.

Stability Study

On study day zero, 57 mL of NS was mixed well with 57 mL of 20 mg/mL dolasetron mesylate (Anzemet, Hoechst Marion Roussel, lot A0D24, expiry October 2002) to prepare 114 mL of a 10 mg/mL solution of dolasetron in NS. Similarly, on the same day, 57 mL of D5W was mixed well with 57 mL of 20 mg/mL dolasetron mesylate (Anzemet, Hoechst Marion Roussel, lot A0D24, expiry October 2002) to prepare 114 mL of a 10 mg/mL solution of dolasetron in D5W. Aliquots (0.5 mL each) of each solution (in NS and D5W) were drawn up into a total of one hundred and ninety-two 3-mL syringes (Monoject, Sherwood Medical, St Louis, Missouri), 96 syringes for each diluent. Half of the prepared syringes (48 with each diluent) were stored at room temperature (23°C) and the other half (48 with each diluent) were stored in the refrigerator (4°C). On each of 12 study days (0, 2, 3, 6, 8, 10, 14, 16, 21, 23, 29 and 31), 4 syringes of each diluent–temperature combination were selected for testing. Samples from 3 of these syringes were subjected to complete chromatographic analysis, and the entire volume of the fourth syringe was expelled into a clean, dry glass test tube for tests of pH and physical appearance.

Dolasetron Analysis

On each study day, standard curves were prepared by diluting 1 mL of 20 mg/mL dolasetron mesylate for injection (Anzemet, Hoechst Marion Roussel, lot A0D24, expiry October 2002) with 9 mL of distilled water to make a 2 mg/mL stock solution. Samples of this stock solution were further diluted with distilled water to obtain standards with final concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mg/mL. These standards and a blank were used to construct the standard curve.

Duplicate 2-µL samples of each standard and the blank were chromatographed. In addition, 2 quality control samples of dolasetron (concentrations 0.625 and 1.125 mg/mL) were chromatographed in duplicate each day; the concentrations of these solutions as determined by chromatography were compared with the known concentrations. Intra-day and inter-day errors were assessed by the coefficients of variation of the peak areas of both quality control samples and standards.

On each of the 12 study days, samples were drawn from the 3 syringes for each diluent–temperature combination, diluted, and then injected directly onto the liquid chromatographic system and assayed for dolasetron content. Injection of duplicate 2-µL samples into the chromatographic system ensured the ability to distinguish concentrations that differed by 10%.[6,7] The concentration of dolasetron in each of the duplicate samples was determined by interpolation from the standard curve of 6 standards, described above. The concentrations were recorded to the nearest 0.001 mg/mL.

Chromatographic Analysis

The liquid chromatographic system consisted of a solvent delivery pump (model P4000, Thermo Separation Products, San Jose, California), which pumped a mixture of 30% acetonitrile (catalogue number AX0142-1, EM Science, Gibbstown, New Jersey) and 70% 0.02 mol/L potassium phosphate monobasic (catalogue number P286, Fisher Scientific, Toronto, Ontario). On each study day, the strength of the mobile phase was prepared to achieve a retention time for dolasetron between 310 and 370 s through a 25 cm x 4.6 mm reverse-phase C_{18}, 5-µm column (Ultrasphere ODS [octadecylsilane] C_{18}; distributed in Canada by Beckman, Mississauga, Ontario) at 2.0 mL/min. Duplicate 2-µL samples were drawn from each of the syringes containing 10 mg/mL of dolasetron and were injected directly onto the column by means of an autoinjector (Ultra WISP 712, Waters Scientific, Toronto, Ontario). The column effluent was monitored with a variable-wavelength ultraviolet detector (Spectra System UV6000LP, Thermo Separation Products) at 230 nm. A signal from the detector was integrated and recorded with a chromatography data system (ChromQuest, Thermo Separation Products). The area under the dolasetron peak at 230 nm was subjected to least-squares linear regression and the actual dolasetron concentration in each sample was determined by interpolation from the standard curve. On the basis of considerations of slope and variability (quantitative resolution) for standards observed during assay validation, dolasetron concentrations were recorded to the nearest 0.001 mg/mL.

Determination of pH

On each of the 12 study days, samples were drawn from one syringe of each diluent–temperature combination, and the pH was measured to the nearest 0.001 pH unit (reported to 0.01 pH unit). The pH meter (Accument model 925, Fisher Scientific, Nepean, Ontario) was standardized on each study day with commercially available buffer solutions.

Visual Inspection

On each of the 12 study days, samples drawn for the purpose of pH measurement from each diluent–temperature combination were inspected visually against a black and a white background to determine the presence of particulate matter, as well as colour and clarity. Samples for visual inspection were removed from the storage container to avoid misinterpretations related to the opacity of the container.

Data Reduction and Statistical Analysis

Data from the accelerated degradation study were analyzed by linear regression and log–linear regression to determine if dolasetron degradation was better described by a first-order or zero-order degradation rate, according to the methods of Box and Cox.[8,9] Means were calculated for replicate analyses and are reported in the summary tables. Mean results from different days for each test (liquid chromatography and pH analyses) were compared statistically to determine if there was an association between the observed result and storage time. Analysis of variance was used to test differences in degradation rate between different diluent–temperature combinations. The 5% level was used as the *a priori* cutoff for significance. Dolasetron concentrations were considered "acceptable" or "within acceptable limits" if the concentration on any day of analysis was greater than 95% of the initial (day zero) concentration and if the percent remaining on day 31, with 95% confidence interval, was greater than 90%.

RESULTS

Accelerated Degradation and Assay Validation

After addition of 1% sodium hypochlorite to a 1 mg/mL dolasetron solution, the concentration of dolasetron declined to 58.06% of the initial

concentration in 110 min. This corresponds to a half-life of 103.6 min under these conditions. These data fit a first-order or pseudo-first-order degradation rate significantly better than a zero-order rate (r = 0.7673 for zero-order rate and 0.9664 for first-order rate). Two degradation products, which eluted at 4.7 and 7.7 min (Fig. 1), were observed in chromatograms from the degraded material. Neither of these degradation products interfered with dolasetron quantification. As a result of the predictable and virtually complete degradation of dolasetron in the accelerated degradation study and the chromatographic separation of the degradation products from dolasetron, it was concluded that this analytical method was suitable for indicating stability.[10,11]

Assay validation was completed with 5 consecutive standard curves. The validation indicated that the dolasetron concentration could be measured accurately and reproducibly. Over the validation period, the deviation of the mean of 2 replicates for each of 6 standards, ranging in concentration from 0.25 to 1.50 mg/mL, was less than 3% from the nominal concentration of every standard and quality control sample. Over the validation period, analytical reproducibility within a day (as measured by the coefficient of variation) averaged less than 2% for each of the 6 standards and 2 quality control samples. After determination of the coefficient of variation of the assay from the results of the prestudy validation, a power calculation indicated that duplicate analysis of each sample had the ability to distinguish between concentrations that differed by at least 6%.[6,7] On the 12 study days, similar accuracy and reproducibility were observed. During the study period, the absolute deviation averaged 1.7% for each of the 6 standards and 2.3% for the quality control samples, and analytical reproducibility within a day (as measured by the coefficient of variation) averaged 1.9% for each of the 6 standards and 1.7% for the 2 quality control samples. Analytical reproducibility between days (as measured by the coefficient of variation) averaged 2.5%.

Stability Study

The temperature of the refrigerator used for storing half of the study samples remained at between 3°C and 4°C throughout the study period.

Table 1 lists the initial measured concentration and the percent remaining over the study period for all dolasetron solutions. All samples retained at least 96.1% of the initial concentration during the study period. The percent remaining on day 31, estimated by linear regression, differed by less than 1.5% from the initial

Table 1. Observed Concentration of Dolasetron in NS and D5W after Storage at 4°C or 23°C*

	4°C		23°C	
Study Day	**NS**	**D5W**	**NS**	**D5W**
0	9.39 ± 0.10	9.24 ± 0.07	9.39 ± 0.10	9.24 ± 0.07
2	102.9 ± 0.8	100.5 ± 2.5	99.9 ± 1.4	101.5 ± 1.4
3	97.4 ± 1.9	100.9 ± 1.7	98.6 ± 1.0	100.1 ± 2.0
6	101.2 ± 0.6	101.9 ± 0.7	97.8 ± 2.8	99.4 ± 2.8
8	104.2 ± 1.3	104.2 ± 1.5	99.7 ± 2.1	101.6 ± 1.5
10	102.4 ± 0.8	103.9 ± 0.4	102.2 ± 2.1	105.8 ± 2.5
14	98.7 ± 1.7	106.2 ± 1.2	101.6 ± 1.2	103.4 ± 0.1
16	103.4 ± 2.7	105.7 ± 1.4	105.7 ± 1.3	104.2 ± 0.6
21	102.2 ± 0.8	103.0 ± 1.0	102.0 ± 1.4	101.4 ± 2.2
23	102.0 ± 2.3	101.9 ± 1.7	101.7 ± 2.8	103.3 ± 0.7
29	100.9 ± 2.0	102.4 ± 2.2	96.1 ± 1.2	96.7 ± 0.3
31	100.7 ± 0.6	100.4 ± 0.5	102.6 ± 1.6	104.0 ± 1.1
Mean % remaining on day 31†	100.6	100.8	101.4	100.7
Lower limit of 95% CI for % remaining on day 31‡	97.6	99.3	96.4	97.0

NS = normal saline (0.9% sodium chloride in water), D5W = 5% dextrose in water, CI = confidence interval.
*Concentrations are expressed as means ± standard deviation, in milligrams per millilitre for day zero and as percent remaining for all other study days. Each mean is based on the analysis of 3 syringes, each analyzed in duplicate.
†The mean percent remaining on day 31 is based on linear regression for each diluent–temperature combination.
‡The lower limit of the 95% CI of the percent remaining on day 31 is based on the slope, calculated by linear regression for each diluent–temperature combination, according to the following formula: 100 x [(concentration on day 0 + (slope x 31 days)] / (concentration on day 0).

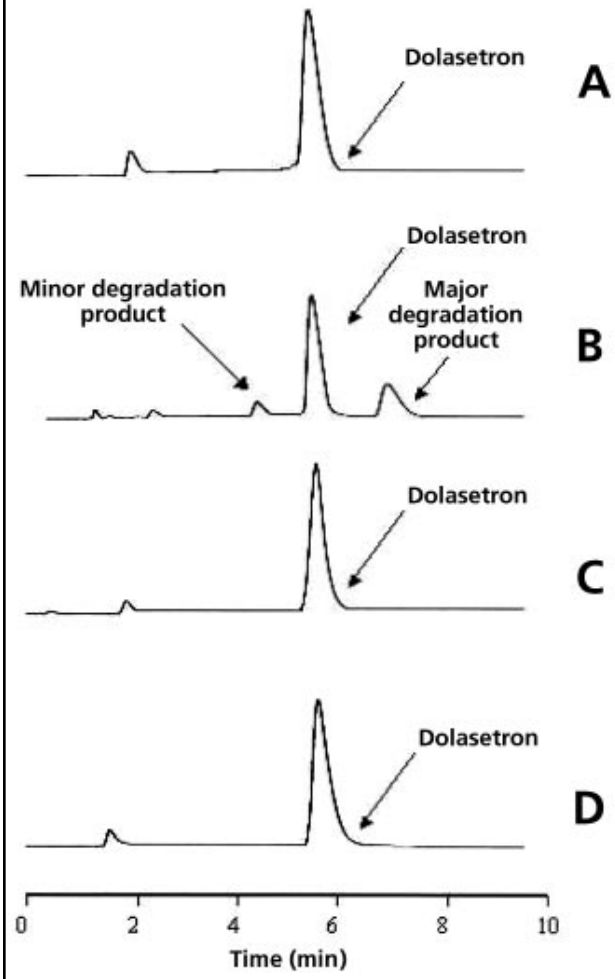

Figure 1. A and B: Chromatograms obtained during the accelerated degradation study of a 1 mg/mL solution of dolasetron in water after 151 min. Chromatogram A represents a sample obtained immediately after the addition of 1% sodium hypochlorite. Chromatogram B represents a sample obtained after 110 min of degradation, when 58.06% of the initial dolasetron concentration remained. Neither of the 2 degradation products, which eluted at 4.7 and 7.7 min, respectively, interfered with quantification of dolasetron, which eluted at 5.9 min. C and D: Chromatograms obtained during the stability study. Chromatogram C represents a 10 mg/mL solution of dolasetron in 0.9% sodium chloride on day zero of the study. Chromatogram D represents the same solution after 31 days of storage at 23°C. No degradation products occurred, and the dolasetron solution retained more than 95% of its initial concentration.

concentration for all diluent–temperature combinations. On the basis of the 95% confidence interval for the slope, the amount remaining on day 31 averaged 100.88% (Table 1). Analysis of variance demonstrated that there was no significant trend for the concentration to change in any diluent-temperature combination. The degradation products observed in the accelerated portion of the study (Figure 1, chromatograms A and B) were not observed in any chromatograms during the stability study.

Table 2. pH of Dolasetron Solutions Prepared NS and D5W and Stored for up to 31 Days

	4°C		23°C (Room Temperature)	
Study Day	NS	D5W	NS	D5W
0	3.68	3.62	3.68	3.63
2	3.79	3.57	3.80	3.64
3	3.80	3.59	3.60	3.72
6	3.63	3.55	3.66	3.60
8	3.75	3.61	3.59	3.69
10	3.65	3.69	3.71	3.70
14	3.59	3.71	3.54	3.56
16	3.61	3.71	3.79	3.51
21	3.59	3.68	3.70	3.59
23	3.76	3.73	3.71	3.72
29	3.74	3.70	3.56	3.74
31	3.59	3.57	3.73	3.51
Change in (pH from day 1 to day 31)	–0.09	–0.06	0.04	–0.12

NS = normal saline (0.9% sodium chloride in water), D5W = 5% dextrose in water.

All solutions were initially clear and colourless and remained so for the duration of the study. No visible particles were observed in any solution throughout the study period. The initial pH of 10 mg/mL dolasetron solutions in NS was 3.68 and ranged from 3.62 to 3.63 in D5W (Table 2). Solutions prepared in NS had a slightly higher pH than solutions prepared in D5W. The pH of all the study solutions did not change significantly over the study period. During storage at both 4°C and 23°C, the pH of all the solutions changed by 0.12 pH unit or less.

DISCUSSION

This study demonstrated that 10 mg/mL solutions of dolasetron in either NS or D5W can be stored at either room temperature or in the refrigerator for at least 31 days with no loss of potency. The authors are unaware of any previously published study dealing with dolasetron stability, other than the information found in the product monograph[4] and the previously published brief report.[5]

Because only small changes in dolasetron concentration were detected under these storage conditions, assurance of the specificity of the analytical method is very important. This specificity was demonstrated by the accelerated degradation portion of the study (Figure 1), during which reductions in the concentration of dolasetron were observed as the concentration of apparent degradation products increased. The separation and detection of intact drug in

the presence of degradation compounds must be assured before the method can be considered suitable for indicating stability,[10,11] and this was accomplished in the current study.

Demonstration of a trend for the concentration to decrease was considered more important than demonstration of statistically significant differences in concentration between any 2 days. In fact, the random fluctuations in concentration around the initial concentration are not of practical importance and should be considered "noise" or experimental error. Linear regression indicated that the concentration on day 31 was within 4% of the initial concentration. Degradation products were not observed in any of the chromatograms, and therefore it is likely that the daily concentrations represented estimates of an unchanging concentration. The inter-day reproducibility error was 2.5% (coefficient of variation expressed as a percentage), which is similar to the error observed during the assay validation of quality control samples and standards.

In conclusion, 10 mg/mL solutions of dolasetron mesylate in NS or D5W, stored in 3-mL polypropylene syringes at 4°C or 23°C, are stable and retain more than 96% of their initial concentration during 31 days of storage.

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