Stability of Extemporaneously-compounded Nadolol 10-mg/mL Suspension in Oral Mix in Glass and Plastic Bottles and Plastic Syringes

Andrea Walsh, PharmD
Blake Ziegler, Honors BSc, PharmD
Karen Lingertat-Walsh, BScPhm, ACPR
Shirley Law, DipPharmTech
Scott E. Walker, MScPhm

Abstract
An oral liquid formulation of nadolol, which is required for administration to patients who cannot swallow intact tablets, is not commercially available. The objective of this study was to evaluate the stability of nadolol 10 mg/mL prepared in Oral Mix vehicle and stored in amber glass, amber polyethylene terephthalate, or amber polyvinyl chloride for 91 days at 4°C and 25°C; and polypropylene oral plastic syringes at 25°C only. Three separate batches of nadolol suspension 10 mg/mL were prepared with Oral Mix. Of the suspension, 50-mL aliquots were stored in 100-mL bottles (amber glass, amber polyethylene terephthalate, or amber polyvinyl chloride). Half of the bottles from each container type were stored at 25°C and the other half at 4°C. On study days 0, 2, 7, 14, 21, 28, 42, 56, 72, and 91, nadolol concentration was determined using a reverse-phase, stability-indicating liquid chromatographic method from samples drawn from each type of container at each temperature. Oral syringes (3 mL), filled with 2 mL of suspension, were stored at 25°C and tested on days 0, 2, 7, 21, 42, and 91. The concentration of nadolol 10 mg/mL in Oral Mix in all study samples from bottles and oral syringes remained within 3.5% of the initial concentration. Based on the fastest degradation rate with 95% confidence, on day 91, between 99% to 100% and 98% to 100% remained in suspensions stored in bottles at 25°C and 4°C, respectively. Oral syringes at 25°C had 94% remaining on day 91. Multiple linear regression analysis demonstrated that the percent remaining was related to study day and container, but not temperature. On day 91, nadolol 10 mg/mL oral suspensions prepared with Oral Mix and stored in all bottle types at 4°C will retain more than 98% of the initial concentration compared to 99% at 25°C and only 94% when stored in oral syringes.

Acknowledgment
This study was funded by an unrestricted educational grant from Medisca, St. Laurent, Quebec, along with the Departments of Pharmacy at Sunnybrook Health Sciences Centre and The Hospital For Sick Children, Toronto, Ontario.

Introduction
Nadolol suspension is not commercially available, yet there is a need for this suspension for patients who cannot swallow tablets. No other published stability data was found.

Objectives
The objective of this study was to determine the physical and chemical stability of 10-mg/mL nadolol suspension in the dye-free vehicle Oral Mix, stored in amber glass, polyvinyl chloride (PVC), and polyethylene terephthalate (PET) bottles, as well as 3-mL clear polypropylene oral syringes for 91 days. Before initiating the nadolol stability study, a physical study on the suspension was conducted to ensure that the formulation did not have any issues when compounded, nor exhibited any undesirable physical characteristics such as caking or clumping over time. This stability study

The authors’ affiliations are: Andrea Walsh, Blake Ziegler, and Karen Lingertat-Walsh, Department of Pharmacy, The Hospital For Sick Children, Toronto, Ontario, Canada; Shirley Law and Scott E. Walker, Department of Pharmacy, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.
follows the same protocol for methodology and results reporting as previously published.1

**Methods**

**FORMULATIONS STUDIED**

A physical study was undertaken to determine the suitability of the nadolol suspension in Oral Mix prior to the stability study. Ease of compounding without any issues were noted. All samples were examined for changes in odor, taste, color, and ease of re-suspension, along with measurement of pH with a Model PHI 510 digital pH meter (Beckman Coulter, Fullerton, California) over 91 days. Bottles were kept at 25°C and 4°C. The refrigerated samples were permitted to equilibrate to room temperature before measurement of pH.

**STABILITY-INDICATING ASSAY DEVELOPMENT AND VALIDATION**

**Liquid Chromatography**

The liquid chromatographic system consisted of an isocratic liquid delivery pump (Model P4000; Thermo Separation Products, San Jose, California), which pumped a mixture of 30% acetonitrile (Lot 55203, Product Number AX0142-1, OmniSolv; Millipore Canada Ltd., Toronto, Ontario) and 70% 0.05M phosphoric acid (Lot L-19621, Product Number A-260, a-phosphoric acid 85%; Fisher Chemical, Fair Lawn, New Jersey) at pH 4.1. On each day, the strength of the mobile phase was prepared to achieve a retention time for nadolol at 4.4 minutes through a 15 cm × 4.6 mm reversed phase LC-18, 3-um column (Catalog 59194, Supelcosil ABZ+Plus; Supelco, Oakville, Ontario, Canada) at 1.0 mL/min. The samples of 5 µL were introduced into the liquid chromatographic system using an auto injector (WISP 712; Waters Scientific, Toronto, Ontario, Canada). The column effluent was monitored with a variable wavelength ultraviolet (UV) detector (UV 6000; Thermo Separation Products, Fremont, California) at 226 nm. A signal from the detector was integrated and recorded with a chromatography data system (ChromQuest 5.0; ThermoQuest Thermo Separation Products; San Jose, California).

**Stability-indicating Methodology**

Following the development of the chromatographic system for nadolol, the suitability of the method for use as a stability-indicating assay was tested by analyzing samples of nadolol that had been subjected to degradation. Accelerated degradation was achieved by an addition of 5 µL of varying strengths of sodium hypochlorite (Lot 096133, sodium hypochlorite 1%, PCS 5000 Oxidizing disinfectant; PCS-Process Cleaning, Peterborough, Ontario), diluted with distilled water to 1.0 mg/mL, to samples of nadolol (Lot BCBH7902v; Sigma Aldrich, Oakville, Ontario) in water, and chromatographed immediately. Sodium hypochlorite (a potent oxidizer) is very effective in degrading drugs at all pH values.

The ultraviolet-visible spectroscopy (UV-VIS) detector is capable of evaluating the UV-VIS spectrum of the chromatographic column effluent every 0.2 seconds. In this way, the UV-VIS spectrum of the effluent can be determined, and the UV-VIS purity of an eluting peak can be evaluated. Changes in the UV-VIS spectrum over the elution profile of the peak of interest would indicate that the peak is contaminated and that the chromatographic separation does not separate nadolol from degradation products and is, therefore, unsuitable. However, if 1) the UV-VIS profile does not change during the elution profile of the peak of interest, 2) the UV-VIS spectrum during the elution profile of the peak of interest is also identical to a sample of known purity (>99%), and 3) the drug of interest can be degraded to a measurable extent while both 1) and 2) remain true during the evaluation of 3), then the chromatographic system will be judged to be stability indicating.1

The chromatograms obtained from each of the degraded nadolol samples were inspected for the appearance of additional peaks. The nadolol peak was compared between samples for changes in concentration, retention time, and peak shape (by means of electronic overlay and numeric calculation of tailing). The UV spectral purity of the nadolol peak in chromatograms of the degraded samples was compared with the spectrum of the authentic un-degraded sample of nadolol obtained at time 0. These procedures met or exceeded published and accepted standards.2-4

**Oral Mix Vehicle and Assay Interference**

A sample of the Oral Mix vehicle, with and without nadolol, was assayed to ensure that there was no interference of the vehicle with the assay.

**Validation of Assay**

Once the specificity of the analytical method was assured, validation for the accuracy and reproducibility of the standard curves was evaluated over a 5-day period. System suitability criteria (theoretical plates, tailing, retention time) were developed to ensure consistent chromatographic performance on each study day.5 On each validation day, 10 mg of a nadolol standard was accurately weighed and diluted into 10 mL of distilled water to make a 1.0-mg/mL stock solution. This stock was further diluted to prepare standard concentrations of 0.094 mg/mL, 0.188 mg/mL, 0.375 mg/mL, and 0.750 mg/mL. The standard curve consisted of these concentrations, the stock solution of 1.0 mg/mL, and a blank. A 5-µL aliquot of each was injected directly into the liquid chromatograph in duplicate. Also, three quality-control (QC) solutions of 0.125 mg/mL, 0.250 mg/mL, and 0.500 mg/mL were prepared on each day and chromatographed in duplicate. The concentrations of the QC samples were determined using the standard curve and were compared to the known concentrations. The range of the calibration curve encompassed the diluted test concentration of nadolol. Within-day and between-day errors were assessed by the coefficients of variation (CV) of the peak areas of both QC samples and standards.
STABILITY STUDY

Nadolol suspensions (10 mg/mL) were prepared with nadolol 80-mg tablets (Lot KG0793; Apotex, Toronto, Ontario) in Oral Mix (Lot 1074/A; Medisca Pharmaceutique Inc., St. Laurent, Quebec). Three separate batches were made (360 mL each). Each suspension batch was then divided into 60-mL aliquots and placed in 6 × 3 separate containers; 6 amber 100-mL glass bottles (Beaton Clark, Rotherham South Yorkshire, England; distributed by Richards Packaging, Mississauga, Ontario); 6 amber PET 100-mL bottles (Eastman Chemical Company, Kingsport, Tennessee; distributed by Jones Packaging), and 6 amber PVC 100-mL bottles (Richards Packaging). A fourth separate batch of suspension was made by Jones Packaging), and 6 amber 100-mL glass bottles (Beatson Clark, Rotherham South Yorkshire, England; distributed by Richards Packaging), and 6 amber PVC 100-mL bottles (Richards Packaging). A fourth separate batch of suspension was made by Jones Packaging), and 6 amber 100-mL glass bottles (Beatson Clark, Rotherham South Yorkshire, England; distributed by Richards Packaging). A fourth separate batch of suspension was made by Jones Packaging), and 6 amber 100-mL glass bottles (Beatson Clark, Rotherham South Yorkshire, England; distributed by Richards Packaging)

Half of the bottles, 3 glass, 3 PET, and 3 PVC plastic bottles, were placed in a refrigerator at 4°C, protected from light. The remaining bottles, 3 glass, 3 PET, and 3 PVC plastic bottles, were stored at 25°C (while exposed to ambient fluorescent light). All syringes were stored at 25°C and were protected from light by storage in a brown UV protective bag. All bottles were partially filled to allow airspace to be above the suspension. These conditions simulate preparation, use, and storage of the suspensions likely to be encountered during clinical practice.

Each test container was shaken well and 0.5 mL was withdrawn from each bottle or syringe immediately following compounding on day 0, and on days 1, 7, 14, 21, 28, 42, 54, 75, and 91. The syringe analysis was completed on days 0, 2, 7, 21, 42, and 91. Each 0.5-mL sample was diluted to 10 mL with methanol (0.5-mg/mL concentration) and mixed well before centrifugation for 10 minutes at 2000 rpm. Then, 5 μL of the supernatant was injected into the HPLC. All aliquots were analyzed in duplicate on the day of sampling using the validated liquid chromatographic system described with UV detection at 226 nm. The area under the nadolol peak at 226 nm was subjected to least-squares linear regression, and the actual nadolol concentration in each sample was determined by interpolation from the standard curve and correction by the dilution factor.

On each study day, standard curves of the same concentrations described under the validation phase were prepared. These five standards and a blank were combined to create a standard curve. The three QC samples (same concentrations as in the validation phase) were also prepared. Each standard and QC sample was chromatographed in duplicate on each study day.

Statistical Analysis

For the assay, within-day and between-day errors were assessed by the CV of the peak areas of both QC samples and standards (during both the assay validation and study periods). After determining the CV of the assay, a power calculation determined that duplicate injection had the ability to distinguish between concentrations which differed by at least ten percent within each individual container.5,6 During the study, means and CVs were calculated for replicated analyses (each sample was assayed in duplicate) of the 3 samples for each container type and temperature. Multiple linear regression analysis was conducted to determine differences in concentration between study days, containers, and temperature.

The percent remaining was analyzed by linear regression and a 95% confidence interval (CI) was constructed around the slope of the curve for percent remaining versus study days. The lower limit of this CI represents the fastest degradation rate with 95% confidence. The time to achieve 90% of initial concentration, using this fastest degradation rate, was the recommended BUD.

Concentrations were considered within acceptable limits if 1) the measured concentration on that study data is greater than 90% of the initial day zero concentration and 2) the concentration on that day, estimated using the fastest degradation rate with 95% confidence, also exceeds 90% of the initial day zero concentration.1

Results

PHYSICAL STUDY

The physical study of nadolol suspensions demonstrated that all suspensions in Oral Mix were uniform, off-white in color, and a bit grainy in texture. During the 91-day study period, no caking or clumping occurred in any suspension stored at either 4°C or 25°C. Some settling was observed to occur between study days, but re-dispersion occurred easily with shaking. All suspensions had a sweet, cherry odor and tasted bitter. The pH of the suspensions stored at 24°C and 25°C in Oral Mix ranged between 6.99 and 7.40 for the duration of the study. The refrigerated samples were permitted to equilibrate to room temperature before measurement of pH.

STABILITY-INDICATING ASSAY

The accelerated study demonstrated that a 1.0-mg/mL concentration of nadolol in water quickly degraded to 3 degradation products when sodium hypochlorite was added to the nadolol solution. Degradation products eluted at 3.5 minutes, 3.7 minutes, and 5.7 minutes, and nadolol eluted at 4.4 minutes (FIGURE 1). Furthermore, Oral Mix did not interfere with the nadolol assay. Since the degradation products do not interfere with nadolol quantification, and as a result of the similarity of the UV spectrum between an authentic nadolol standard and nadolol in a degraded sample, it was concluded that this analytical method was stability indicating.

ASSAY VALIDATION AND PERFORMANCE

During the assay validation, regression analysis of the peak area ratio of nadolol versus the concentration of each nadolol standard was best described by a polynomial over the concentration range, with coefficient of determination (r²) >0.9998 (n=5). Analysis of standard curves and QC samples indicated that the nadolol concentrations were measured accurately and reproducibly. Within-day analytical reproducibility (as measured by CV) averaged 0.90% for the standard concentrations and 1.20 for the QC samples.
Between-day analytical reproducibility (as measured by CV) averaged 1.00% for the standard concentrations and 1.62% for the QC samples. Accuracy, based on the absolute deviation from the known concentration, averaged 1.16% for the standards and 2.35% for the QC samples.

ASSAY ANALYSIS DURING THE STABILITY STUDY

Similarly, during the study period, regression analysis of the peak area ratio of nadolol versus the concentration of each nadolol standard produced standard curves best described by a polynomial with coefficient of determination ($R^2 > 0.999$ ($n=10$)).

Within-day variation reproducibility (as measured by CV) averaged 1.45% for the standard concentrations and 1.50% for the QC samples. Between-day analytical reproducibility (as measured by CV) averaged 1.48% for the standard concentrations and 2.44% for the QC samples. The between-day variation in nadolol concentration as assessed by the standard deviation of regression, averaged 1.69% for study samples. Accuracy, based on the absolute deviation from the known concentration, averaged 1.90% for the standards and 2.84% for the QC samples.

Therefore, nadolol was measured accurately and reproducibly on each day of the study. This indicates that differences of 10% or more could be confidently detected within individual containers.

Chemical Stability and Statistics
The percent remaining observed on each day during the study period is listed in Table 1. The concentration of nadolol in Oral Mix in all study samples from bottles and oral syringes remained above 96.5% of the initial concentration for 91 days when stored in 3 types of containers (amber glass, PET, PVC) at 2 different temperatures (25°C and 4°C) and in oral syringes at 25°C (Table 1). In fact, even on close inspection of Table 1, very little difference can be seen between containers and temperatures. Nevertheless, multiple linear regression analysis demonstrated that the percent remaining was related to study day ($P<0.001$) and container ($P< 0.033$), but not temperature ($P<0.247$). Although the difference in containers is significant, the difference in percent remaining on day 91, with 95% confidence, is only 0.59% at room temperature and 3.06% at 4°C.

Discussion
This study has demonstrated that a 10-mg/mL nadolol suspension could retain stability of more than 96.5% of the initial concentration when stored for 91 days in amber glass, PVC, and PET bottles, as well as clear polypropylene oral syringes. Even so, after calculation of the 95% confidence limits observed in this study, we recommend a BUD on suspensions stored at 4°C or 25°C not to exceed 91 days.

Inspection of Table 1 will reveal that all changes in percent remaining (slope) are positive (between 0.002% per day and 0.06% per day). This can occur for a number of reasons such as: assay variability due to instrumentation; variation in the accuracy of dilution; sampling technique with suspensions; and water loss from the suspension over time. While the first two reasons can cause random variation leading

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Container Type</th>
<th>Temperature</th>
<th>Percent Remaining</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amber Glass</td>
<td>25°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
<tr>
<td>PET</td>
<td>25°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
<tr>
<td>PVC</td>
<td>25°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
<tr>
<td>Amber Glass</td>
<td>4°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
<tr>
<td>PET</td>
<td>4°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
<tr>
<td>PVC</td>
<td>4°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
<tr>
<td>Oral Syringes</td>
<td>25°C</td>
<td>&gt;96.5%</td>
<td>1.00%</td>
</tr>
</tbody>
</table>

---

**FIGURE 1.**

**CHROMATOGRAMS OF NADOLOL.**

A. Chromatogram A represents 1-mg/mL nadolol in water. Chromatogram B represents 1-mg/mL nadolol with 0.5% sodium hypochlorite (72% of the initial nadolol concentration remaining). Chromatogram C represents 1-mg/mL nadolol in water with 1% sodium hypochlorite added (only 23% of the initial nadolol concentration remaining). Three degradation products are observed, and the degradation products eluted at 3.5 minutes, 3.7 minutes, and 5.7 minutes.
TABLE 1.
NADOLOL CONCENTRATIONS¹ AND PERCENT REMAINING¹ ON EACH STUDY DAY AND CALCULATION OF THE TIME TO ACHIEVE 90% REMAINING (T-90) WITH 95% CONFIDENCE.

<table>
<thead>
<tr>
<th>STUDY DAY</th>
<th>PVC 4°C</th>
<th>GLASS 4°C</th>
<th>PET 4°C</th>
<th>PVC RT</th>
<th>GLASS RT</th>
<th>PET RT</th>
<th>SYRINGE RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10.7±2.14</td>
<td>10.7±0.40</td>
<td>10.6±2.84</td>
<td>10.7±2.09</td>
<td>11.0±0.38</td>
<td>10.5±2.18</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.0532</td>
<td>0.0025</td>
<td>0.0397</td>
<td>0.0272</td>
<td>0.0334</td>
<td>0.0513</td>
<td>0.0596</td>
</tr>
<tr>
<td>14</td>
<td>99.78±2.19</td>
<td>99.57±1.43</td>
<td>99.76±0.31</td>
<td>97.71±4.90</td>
<td>98.45±1.52</td>
<td>97.16±4.33</td>
<td>100.27±0.50</td>
</tr>
<tr>
<td>56</td>
<td>100.9±2.85</td>
<td>99.71±1.18</td>
<td>99.84±2.16</td>
<td>99.34±1.26</td>
<td>100.23±1.63</td>
<td>98.49±2.81</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>102.58±1.05</td>
<td>98.51±2.51</td>
<td>99.75±0.57</td>
<td>98.18±3.02</td>
<td>101.55±1.81</td>
<td>102.02±0.96</td>
<td>104.88±3.82</td>
</tr>
<tr>
<td>2</td>
<td>101.96±6.82</td>
<td>97.65±4.49</td>
<td>97.28±2.62</td>
<td>100.24±0.88</td>
<td>100.72±2.29</td>
<td>102.39±1.58</td>
<td>108.35±1.56</td>
</tr>
<tr>
<td>72</td>
<td>102.43±1.96</td>
<td>103.67±3.83</td>
<td>101.76±2.79</td>
<td>101.25±2.27</td>
<td>103.43±1.96</td>
<td>102.16±3.24</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>103.16±2.58</td>
<td>102.43±1.96</td>
<td>103.93±3.72</td>
<td>102.39±1.58</td>
<td>103.43±1.96</td>
<td>102.16±3.24</td>
<td></td>
</tr>
</tbody>
</table>

1. Concentrations are expressed as the percent remaining ± the standard deviation. Percent remaining is calculated based on the concentration determined in duplicate of each of the three replicate vials stored at each temperature relative to the concentration on study day zero.

2. The degradation rate [slope] was determined by linear regression of the percent remaining on each study day.

3. Sy.x is the standard deviation of regression. This is equivalent to the interday variability (error) of the analytical method, expressed as a percent.

4. The Confidence Interval for the Degradation Rate allows calculation of the Fastest and Slowest Degradation Rate with 95% Confidence (Degradation Rate ± Confidence Interval).

5. The T-90 is the time for the concentration to decline by 10% (i.e., to achieve 90% of the initial concentration). The shortest T-90 uses the Fastest Degradation Rate determined from the 95% confidence limit of the slope.

PET = polyethylene terephthalate; PVC = polyvinyl chloride; RT = room temperature

1. To concentration estimates on any given day greater than 100%, consistent and constant increases in concentration are more likely to be due to water loss. Water loss has been reported previously with PVC bags,⁶ CADD Reservoirs, PVC bags, and ethylene/propylene co-polymer (PAB) bags.⁸-¹⁰ We believe water loss is the most likely explanation since day-to-day variability, as measured by the standard deviation of regression, averaged 1.69% across all temperatures and containers (including syringes).

Although the data indicates that nadolol stability would be longer than 90 days, the BUD should not be extrapolated past the last day in a stability study.¹ The syringes produced a calculated BUD with 95% confidence, which was 94.5% on day 91. Because there was no significant difference in concentration between each of the tested temperatures and container combinations, we recommend the shortest BUD estimated to be 91 days following preparation.

When a drug is stored under controlled lab conditions, which stability studies are, the concentrations on each study day will be determined by 1) the initial concentration, 2) the degradation rate, 3) the analytical variability, and 4) the duration of storage.¹ During a study, there will be some variability in these.
Concentrations due to error in the analytical method (accuracy and reproducibility). Therefore, daily concentrations reported in a stability study should be viewed only as random estimates of the true concentration on that study day.\textsuperscript{1} As a result of this variability around the true concentration, the degradation rate is then best calculated using linear regression. The slope of this line is the degradation rate, and it will have units of percent per day when the data is presented as percent remaining.\textsuperscript{1}

However, we still need to be assured that once this product is compounded using the same methodology and stored for the same amount of time, the concentration of the drug is not less than 90\% of the initial concentration. Acknowledgement that analytical variability is inherent in each study, the “best estimate” of the degradation rate should be made using the lower limit of the 95\% CI of the linear regression line.\textsuperscript{1}

Specificity of the analytical method used during the study is very important, especially when there is only a small change in drug concentration, as seen with nadolol. Furthermore, the accuracy and reproducibility of the assay in the validation phase and on every assay day during the study provides the necessary confidence in the methodology used.\textsuperscript{1}

Even though Richards Packaging no longer sells PVC bottles, we did obtain a supply for this study, as PVC may still be available in other countries.\textsuperscript{1}

\section*{Conclusion}

The amount of 10-mg/mL nadolol suspension remaining after 91 days of storage with 95\% confidence in all bottle types at 4°C was more than 98\%; at 25°C, it was more than 99\%; and, in syringes (25°C), it was more than 94\%. Since most pharmacies would like to store nadolol suspension at 25°C for convenience, it is suggested that nadolol can be made in PET, PVC, glass bottles, or polypropylene syringes and assigned a BUD of 91 days.

\section*{References}


Address correspondence to Karen Walsh, Compounding Resource Pharmacist, Department of Pharmacy, The Hospital For Sick Children, 555 University Avenue, Toronto, Ontario. Canada, M5G 1X8. E-mail: Karen.Walsh@sickkids.ca