Stability of Melatonin in an Extemporaneously Compounded Sublingual Solution and Hard Gelatin Capsule

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ABSTRACT
This study examined the stability of melatonin in a 10-mg/mL oral sublingual solution stored at 4°C or 25°C and in 3-mg capsules stored at ambient (25°C; 60% relative humidity) and accelerated (40°C; 75% relative humidity) conditions over a period of 90 days. A sublingual solution of melatonin 10 mg/mL was prepared with glycerin, ethyl alcohol, stevia powder extract, and tutti-frutti flavor. Six identical solutions were prepared and stored in prescription amber glass bottles at 4°C or 25°C. Triplicate 1-mL samples from each of the six solutions were assayed immediately after preparation and after 7, 14, 28, 60, and 90 days with a stability-indicating high-performance liquid chromatographic method. Six batches of 100 melatonin 3-mg capsules were prepared with Methocel E4M and lactose anhydrous and stored in prescription amber glass bottles at ambient or accelerated conditions. A sample of 10 capsules from each batch was assayed immediately after preparation, and additional samples from each storage condition were assayed at 7, 14, 28, 60, and 90 days. The mean concentration of melatonin exceeded 98% of the initial concentration throughout the 90-day study period for the sublingual solution and capsules under all storage conditions. There were no detectable changes in color, odor, taste, or pH, and no visible microbial growth in any of the sublingual solution samples. Compendia requirements for content uniformity were met for the extemporaneously prepared capsules. Melatonin in an extemporaneously compounded sublingual solution (10 mg/mL) was stable for at least 90 days when stored in prescription amber glass bottles at 4°C or 25°C. Melatonin in extemporaneously prepared capsules (3 mg) was stable for at least 90 days when stored in prescription amber glass bottles at ambient or accelerated conditions.

INTRODUCTION
Melatonin is a naturally occurring hormone produced by the pineal gland in the brain.1 Melatonin supplementation has been shown to be effective in travelers suffering from jet lag, in restoring sleep patterns in the elderly, and for the treatment of insomnia and sleep cycle disorders in children with neurodevelopmental disabilities or psychiatric disorders.2-3 Melatonin has also been shown to be a potent antioxidant and free radical scavenger with beneficial effects in preventing and treating Alzheimer’s disease.4 Melatonin is not commercially available in Australia and is prepared extemporaneously in a number of dosage forms in both community and hospital practice. To date, there are no data available regarding the stability of extemporaneously compounded melatonin-containing preparations.

MATERIALS AND METHODS
Sample Preparation
Sublingual Solution
A solution of melatonin 10 mg/mL was prepared (formula-
tion supplied in sidebar) by dissolving 1000 mg of commercially available melatonin powder (Lot 200703; Bella Corporation, Ashmore, Queensland, Australia) and 250 mg stevia powder extract (Lot 07-00018-E; Professional Compounding Centers of America [PCCA], Matraville, New South Wales) in 15 mL United States Pharmacopeia (USP) ethyl alcohol 190 proof (Lot 202128; David Craig, Laverton North, Victoria). Tutti-frutti flavor (2 mL;
to a final volume of 100 mL. Six identical batches of this solution were placed in 100-mL amber glass prescription bottles (Plasdone, Virginia, Queensland). Three of the solutions were refrigerated at 4°C ± 1°C, and three were stored at accelerated conditions (25°C ± 1°C; 60% ± 1.5% relative humidity [RH]) in an climatic chamber compliant with the guidelines of the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (Angelaton; CH1700V; Biolab, Mulgrave, Victoria) as per ICH guidelines.7 Triplicate 1-mL samples were withdrawn from each of the solutions immediately after preparation and 7, 14, 30, 60, and 90 days after preparation. After further dilution of these samples to an expected concentration of 0.1 mg/mL with a dissolution solution (0.05 M KH2PO4, pH 3; Lot AF612289; Ajax Finechem, Seven Hills, New South Wales), the samples were filtered with 0.45-μm Fluoropore membrane filters (Lot R6KN81290; Millipore, Carrigtwohill, Ireland) and assayed against standards as per the high-performance liquid chromatography (HPLC) method described below. All solutions were observed for changes in color, odor, and taste; the pH, detected with the use of a pH meter from Hanna Instruments (Model HI931000; Woosocket, Rhode Island) of each solution was measured on each study day.

**Hard Gelatin Capsules**

Melatonin 3-mg capsules were prepared (formulation supplied in sidebar) by mixing through geometric dilution 300 mg melatonin powder (Lot 20070211; Bella Corporation), 10 mg food color powder (Lot C112270; PCCA), 10.0 g Methocel E4M (Lot 07-000060-9; PCCA), and 20.0 g lactose anhydrous (Lot 07-00015-1; PCCA) in a glass mortar and pestle. One hundred hard gelatin capsules (size 1; Lot 06170511; Bella Corporation) were then prepared with this powder blend using a proprietary capsule machine (Janssen 100; PCCA). Six identical batches of capsules were placed in 100-mL amber glass prescription bottles. Three of the batches were stored at ambient conditions (25°C ± 1°C, 60% ± 1.5% RH), and three were stored at accelerated conditions (40°C ± 1°C, 75% ± 1.5% RH) in ICH-compliant climatic chambers as per ICH guidelines.7 The contents of 10 capsules from each batch were quantitatively transferred to a 500-mL volumetric flask and diluted with the dissolution solution (0.05 M KH2PO4, pH 3) to provide a final theoretical concentration of 0.06 mg/mL, and filtered. These solutions were assayed immediately after preparation and at 7, 14, 30, 60, and 90 days per the HPLC method. Capsules were observed for changes in gross physical appearance or consistency, including hardening or softening of the gelatin shell, and were individually weighed on each study day. Content uniformity8 of the dosage units was determined for each of the six batches at time zero by quantitatively transferring the individual contents of each of the 10 capsules into a 50-mL volumetric flask and diluting with the dissolution solution to provide a final theoretical concentration of 0.06 mg/mL. The samples were then filtered and assayed as per the HPLC method. Samples were protected from light at all times.

**HPLC Method**

The HPLC instrumentation included a solvent delivery module with degasser (LC-20AD, DGU-20A; Shimadzu, Kyoto, Japan), an autosampler (SIL-20AC; Shimadzu), a photodiode array detector (SPD-M20A; Shimadzu), a chromatography data system (Class-VP614; Shimadzu), and a C18 reverse-phase column (Lot 3506, Prevail C18 [3 mm, 150 x 2.1 mm; Grace, Deerfield, Illinois]) maintained at room temperature (23°C). Mobile phase was prepared by combining phosphate buffer (0.015 M KH, PO4; Lot AF612289; Ajax Finechem) containing 0.25% triethylamine (pH 2.4; Lot ZA 3532840-203; BDH, Poole, England), methanol (Lot K37654007-731; Merek, Darmstadt, Germany), and acetonitrile (Lot I3774230-728; Merek) in the proportions 67:19:14, respectively. A detection wavelength of 223 nm was used, the flow rate was 1.0 mL/min, and the injection volume was 20 μL. Concentrations of melatonin in the samples were determined from respective peak areas in relation to constructed standard curves and then converted to a percentage of the initial melatonin concentration. Suitability of the system was checked prior to use on each day of use.

**Rx**

**MELATONIN 10-MG/ML SUBLINGUAL SOLUTION**

For 100 mL

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Stevia powder extract</td>
<td>250 mg</td>
</tr>
<tr>
<td>Ethyl alcohol 190 proof</td>
<td>15 mL</td>
</tr>
<tr>
<td>Tutti-frutti flavoring</td>
<td>2 mL</td>
</tr>
<tr>
<td>Glycerin</td>
<td>qs</td>
</tr>
<tr>
<td></td>
<td>100 mL</td>
</tr>
</tbody>
</table>

**Note:** Melatonin is light sensitive, and thus exposure to light should be minimized.

**METHOD OF PREPARATION**

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Weigh and/or measure each ingredient accurately.
3. Mix 1000 mg melatonin powder with 250 mg stevia powder extract thoroughly in a mortar and pestle.
4. Add the powder blend to a measure. Rinse the mortar and pestle with 7.5 mL of ethyl alcohol 190 proof and add to the same measure.
5. Add a further 7.5 mL of ethyl alcohol 190 proof to the measure and stir until completely dissolved.
6. Add 2 mL tutti-frutti flavor to the solution.
7. Add sufficient glycerin to bring the final volume to 100 mL.
8. Transfer the solution, after thorough mixing, to an amber glass prescription bottle.
9. Label.

**STABILITY**

A beyond-use date of 90 days can be used for this preparation.

**LABELING**

Shake well. Refrigerate.
**Assay Validation**

Method validation for linearity, specificity, and precision was carried out as per ICH guidelines. A calibration curve for melatonin was constructed for concentrations from 0.0100 mg/mL to 0.3 mg/mL using a 1 mg/mL melatonin reference standard (Lot 065K1239; Sigma Aldrich, Sydney, Australia).

Peak purity was assessed by visual comparison with reference material via overlaid spectra obtained on the peak up slopes (20% maximum absorbance), peak maxima, and peak down slopes (20% of peak maxima). Peak purity was further examined by comparing the peak purity parameters obtained at the same three points across the melatonin chromatographic peak for the reference material, with those obtained after forced degradation of the melatonin 10-mg/mL sublingual solution. The extemporaneous sublingual solution was subjected to forced degradation by exposure to (1) acid (0.1 N hydrochloric acid; Ajax Chemicals, Sydney, New South Wales); (2) base (0.1 N sodium hydroxide; Lot AF411397; Ajax Finechem); (3) oxidation (0.3% hydrogen peroxide; Lot 62370; Sigma Aldrich), each at 60°C for 2 days; and (4) light (visible wavelength: 400 to 800 nm at 2.4 million lux hours and ultraviolet.

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**Rx**

**MELATONIN 3-MG CAPSULES**

*For 100 capsules*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin</td>
<td>300 mg</td>
</tr>
<tr>
<td>Food color powder</td>
<td>10 mg</td>
</tr>
<tr>
<td>Methocel E4M</td>
<td>10 g</td>
</tr>
<tr>
<td>Lactose anhydrous</td>
<td>22 g</td>
</tr>
<tr>
<td>Capsules, size 1</td>
<td>100</td>
</tr>
</tbody>
</table>

*Note: Melatonin is light sensitive, and thus exposure to light should be minimized.*

**METHOD OF PREPARATION**

1. Calculate the required quantity of each ingredient for the total amount to be prepared.
2. Weigh and/or measure each ingredient accurately.
3. Add small amounts of lactose anhydrous and food color to a mortar and pestle and mix well.
4. Add the remaining powders and mix thoroughly using the principles of geometric dilution. *Note: All particles should be reduced to the same size.*
5. Prepare 100 capsules (size 1) with the powder blend according to the operating procedures for the Jaansun 100 capsule machine.
6. Transfer the required number of capsules to an amber glass prescription bottle.
7. Label.

**STABILITY**

A beyond-use date of 90 days can be used for this preparation.

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**Figure.** Typical chromatograms of melatonin standard (A), melatonin sublingual solution (B), and sublingual solution blank vehicle (C), and a composite chromatogram of melatonin sublingual solution after forced degradation by heat, acid, base, oxidation, and light (D).
Table 1. Stability of Melatonin in a 10-mg/mL Sublingual Solution and 3-mg Hard Gelatin Capsules.

<table>
<thead>
<tr>
<th>Dosage Form and Storage Temperature</th>
<th>Initial Concentration (mg/mL)x</th>
<th>Initial Concentration Remaining (percentage)x</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 4°C</td>
<td>9.83 ± 0.10</td>
<td>Day 7: 101.2 ± 1.2</td>
</tr>
<tr>
<td>A: 25°C; 60% RH</td>
<td>9.28 ± 0.07</td>
<td>Day 14: 104.4 ± 0.7</td>
</tr>
<tr>
<td>B: 25°C; 60% RH</td>
<td>2.76 ± 0.30</td>
<td>Day 28: 101.8 ± 0.7</td>
</tr>
<tr>
<td>B: 40°C; 75% RH</td>
<td>2.82 ± 0.30</td>
<td>Day 60: 101.2 ± 1.9</td>
</tr>
<tr>
<td>A = melatonin sublingual solution; B = melatonin hard gelatin capsules</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± standard deviation (n = 3)

Analysis of Data
Stability of melatonin in the pharmaceutical preparations was determined as the percentage of the initial concentration remaining at each test interval and defined as retention of greater than 90% of the initial concentration of melatonin.

RESULTS AND DISCUSSION
The retention time for melatonin was 5.3 minutes (Figure). Linearity was confirmed over the concentration range used (r² = 0.999). Visual examination of the overlaid spectra on the peak upstroke, maximum, and downstroke, when compared to the reference material, showed no signs of interfering substances in the melatonin peak under any of the forced degradation conditions. The absence of co-eluting degradants and excipients was verified with peak purity parameter values above 0.999 at all three points across the chromatographic peak for all samples exposed to forced degradation. A study by Andrisano et al. of the photostability of melatonin using HPLC with diode array detection demonstrated that the main degradant of melatonin, because of exposure to light and oxygen, has a substantially different spectrum than melatonin, suggesting that spectra obtained by diode array will demonstrate differences from a standard if photodegradation has occurred. The coefficient of variation for the reference material prepared on five separate occasions was 1.4%, and the coefficients of variation for the solutions with spiked concentrations of 0.05 and 0.2 mg/mL were less than 0.4% for both concentrations over 10 weeks.

The percentages of melatonin remaining in the sublingual solution after 90 days were 101.0% ± 0.8% and 99.8% ± 0.2% at refrigerated (4°C) and accelerated (25°C; 60% RH) conditions, respectively (Table 1). There were no detectable changes in color, odor, or taste and no visible growth in any sample. Throughout the study, the apparent pH values for the sublingual solutions changed <0.5 pH unit from the initial value of 3.2.

Table 2. Content Uniformity of 3-mg Melatonin Capsules at Time Zero.

<table>
<thead>
<tr>
<th>Batch Number</th>
<th>Mean (n = 10)x</th>
<th>SDx</th>
<th>AVex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94.4</td>
<td>4.0</td>
<td>13.6</td>
</tr>
<tr>
<td>2</td>
<td>93.7</td>
<td>2.7</td>
<td>11.3</td>
</tr>
<tr>
<td>3</td>
<td>94.1</td>
<td>3.3</td>
<td>12.3</td>
</tr>
<tr>
<td>4</td>
<td>95.6</td>
<td>2.4</td>
<td>8.8</td>
</tr>
<tr>
<td>5</td>
<td>90.3</td>
<td>2.3</td>
<td>13.8</td>
</tr>
<tr>
<td>6</td>
<td>92.7</td>
<td>1.4</td>
<td>9.1</td>
</tr>
</tbody>
</table>

*Mean of individual capsule contents expressed as a percentage of the label claim
†Sample standard deviation (n = 10)
‡Acceptance value; maximum allowed, 15.0x

The percentages of melatonin remaining in the hard gelatin capsules after 90 days were 103.2% ± 0.8% and 100.6% ± 3.7% at ambient (25°C; 60% RH) and accelerated (40°C; 75% RH) conditions, respectively (Table 1). There were no detectable changes in capsule consistency, including hardening or softening of the capsule shell, with no appearance of fog or liquid droplets or clumping of the capsules inside the container, and no significant difference (P>0.05) in weight variation throughout the study. The United States Pharmacopeia requirements for uniformity of dosage units were met for the melatonin capsules, with acceptance values for the content uniformity tests falling within the defined limits (Table 2).

CONCLUSION
The assay method presented is suitable for use in stability studies of melatonin sublingual solution and gelatin capsule preparations. Melatonin in an extemporaneously compounded sublingual solution (10 mg/mL) was stable for at least 90 days when stored in prescription amber glass bottles at 4°C or 25°C. Melatonin in extemporaneously prepared hard gelatin capsules (3 mg) was stable for at least 90 days when stored in prescription amber glass bottles at 25°C or 40°C conditions. It is important to use appropriate storage containers that protect the preparations from light and moisture and to counsel patients on appropriate storage locations. Although the melatonin in the sublingual preparations is in solution, it is recommended that patients and caregivers shake the bottle before administering a dose, especially if the solution is stored under refrigerated conditions.

REFERENCES


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### Scientific and Professional Reviewers for 2008

The International Journal of Pharmaceutical Compounding (IJP) expresses its sincere appreciation for the efforts of its scientific and professional reviewers for the year 2008. If we failed to acknowledge any 2008 reviewer, we apologize. Most of these reviewers also serve as reviewers for other journals, so we are especially grateful for their time, efforts, and interest in helping IJP maintain a high standard of excellence.

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