RESEARCH ARTICLE

Evaluation of stability of melatonin in extemporaneously compounded oral suspensions

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Abstract

Background: Melatonin is commonly used to treat insomnia, sleep disturbance or hyperactivity disorders. It can be administered in paediatric patients as oral formulations prepared using either bulk powder or commercial products. Few studies have evaluated the stability of these oral formulations, but some storage conditions information may be lacking and a need has been identified for additional stability studies.

Aim: The aim of this research was to study the stability of extemporaneously prepared oral suspensions of melatonin (2 mg/mL), prepared from pure drug powder or commercial tablets using a proprietary dye-free oral vehicle: Oral Mix SF (Medisca Pharmaceutique Inc.).

Method: Two melatonin oral liquid preparations were formulated: (a) tablets in Oral Mix SF and (b) pure drug powder in Oral Mix SF. Preparations were stored at room temperature (23–27°C) as well as under refrigeration (3–5°C) in amber plastic syringes (Precise-Dose Dispenser®), Medisca Pharmaceutique Inc.) and amber polyethylene terephthalate (PET) bottles with appropriate closures for a period of 90 days. Preparations were assessed using a validated stability-indicating high-performance liquid chromatography method. Beyond use dates were evaluated by statistical analysis of the overall degradation trend.

Results: All preparations were physically and chemically stable for at least 90 days in all storage conditions at room temperature and under refrigeration.

Conclusion: Results showed that compared to other vehicles, these suspensions had equivalent stability, indicating that Oral Mix SF is a viable dye-free and sugar-free alternative for extemporaneous compounding of melatonin.

Keywords: stability, pharmacy practice, pharmaceutical stability.

INTRODUCTION

Pharmaceutical compounders create customised drug treatments to ensure that the individual needs of a patient are met. Due to the unique properties of the tailored drug treatment, each formulation will have different stabilities.1 Said stability will depend on the specific properties of the active pharmaceutical ingredient (API) as well as the other ingredients used.2 As a result, stability testing should be performed in order to establish the quality of the drug product over time.

A common form of compounding is the delivery of an active chemical in a liquid dosage form as opposed to a solid form. Oral liquids are commonly used with paediatric and geriatric patients who are unable to swallow commercially available tablets.3 According to the United States Pharmacopeia chapter 795, in the absence of stability studies on a compound, a beyond use date (BUD) of 14 days is suggested for aqueous oral preparations.4 Furthermore, the sample must be stored between 2-8°C. These restrictions can be costly and time-consuming for both the patient and the pharmacist. Furthermore, this stability assumption is certainly not true for all products. However, with empirical stability results on a compounded formulation, a pharmacist can formulate high-quality medication for the duration of treatment and can save time and resources on preparation. The field of pharmaceutical compounding is growing, and with it grows the need for empirical studies on the stability of compounded formulations.2

Melatonin is often extemporaneously compounded. Therefore, related stability data is required for these preparations. Melatonin is a compound that regulates the circadian rhythms, including the sleep-wake cycle.5,6 It is commercially available most commonly in tablet

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form, either as extended-release or sublingual tablets. The available dosage forms are 1, 3 and 5 mg tablets. It is also available as intraroral sprays and oral liquids containing alcohol. Melatonin is commonly used as a sleep aid to modulate sleeping patterns.\textsuperscript{6,7} Additionally, it is sometimes used to slow the advancement of neurodegenerative diseases.\textsuperscript{6,7}

This study is aimed at establishing the chemical stability of extemporaneously prepared melatonin formulations using proprietary Oral Mix SF (Medisca Pharmaceutique Inc, Montreal, QC, Canada). Oral Mix SF is a dye-free, cherry-flavoured oral suspending vehicle used for extemporaneous compounding. Oral Mix is composed of purified water, glycerin, sorbitol, flavoring, microcrystalline cellulose, carboxymethylcellulose sodium, sodium saccharin, xanthan gum and carrageenan, buffered with sodium citrate and citric acid and preserved with potassium sorbate, methylparaben and propylparaben. It is also sugar-free, thus rendering it suitable for patients with dietary restrictions.

METHODS

In order to evaluate the chemical and physical stability of melatonin, 10 mg melatonin tablets (Adrien Gagnon, La Prairie, QC, Canada) and melatonin pure drug powder (Medisca Pharmaceutique Inc, Montreal, QC, Canada) were used to prepare oral formulations (2 mg/mL) with Oral Mix SF as a vehicle. Pure melatonin powder (500 mg) was accurately weighed and mixed in a mortar with the vehicle (1 mL) until it formed a smooth paste. Additional amounts of the vehicle were then incorporated in increments, and the resultant mixture was transferred to a graduated cylinder. The preparation was brought to the required final volume (250 mL) with additional vehicle and thoroughly mixed, resulting in a 2.0 mg/mL concentration. Similarly, melatonin tablets (50 × 10 mg tablets) were crushed using a mortar and a pestle. The powder was mixed with a small amount of vehicle (10 mL) to form a homogeneous paste. Additional vehicle was added in increments up to the required final volume (250 mL) and thoroughly mixed as previously described.

To study the influence of storage containers on the stability of melatonin, preparations were packaged in either 50 mL amber polyethylene terephthalate (PET) bottles with black phenolic caps (filling volume of 30 mL) or 1 mL amber plastic syringes with tip caps (filling volume of 1 mL), both provided by Medisca Pharmaceutique Inc. A total of six bottles and 48 plastic syringes for each type of formulation were prepared.

Samples were stored under refrigeration (3–5°C) and at room temperature (23–27°C/55–65% relative humidity (Forma Environmental Chamber, Thermo Scientific, Cleveland, OH, USA). A 1 mL sample of each tested preparation was taken at each study time point for high-performance liquid chromatography (HPLC) analysis. Prior to sampling, bottles were shaken and the suspensions inspected visually for consistency, color and odour changes. In the case of syringes, the organoleptic properties were verified after the transfer of suspensions into 1.5 mL centrifuge tubes. The pH of each sample was evaluated at time zero and after 7, 14, 30, 45, 60, 75 and 90 days, using a pH meter (pH 211, Hanna Instruments, Montreal, QC, Canada).

Reagents and Chemicals

Melatonin drug powder and the Oral Mix SF vehicle used in this study were provided by Medisca Pharmaceutique Inc. Melatonin 10 mg tablets (Adrien Gagnon) were also used. Acetonitrile and methanol were HPLC grade and were obtained from Fisher Scientific. All water was deionised (18 MΩ resistivity).

Chromatographic System

The quantification of the drug was performed using a reversed-phase HPLC system (Prominence UFLC, Shimadzu Scientific Instruments, Kyoto, Japan) comprising an LC-20 AD binary pump, a DGU-20A5 solvent degasser, an SPD-M20A multiple wavelength photodiode array detector (PDA), an SIL-20 AC HT refrigerated autosampler and a CTO-20 AC column oven.

The flow rate was 1.0 mL/min, and the injection volume was 5 μL. The detector wavelength was set at 220 nm. The separation was performed at 40°C on a Kinetex XB C18 (4.6 × 100 mm, 5 μm, Phenomenex, Torrance, CA, USA) column. An isocratic mode of 7 min was applied where the composition of the mobile phase was 30% methanol and 70% water.

Calibration Curve

In order to prepare a calibration curve for the vehicle (Oral Mix SF), 40 mg of melatonin was suspended in 20 mL of each vehicle to obtain a 2 mg/mL stock suspension. Six hundred microlitres of the stock suspension were mixed with 1800 μL acetonitrile in order to precipitate the vehicle excipients. The mixture was vigorously mixed using a vortex for 20 s and then centrifuged at 12 000 g for 15 min. The supernatant was recovered and used as a stock solution. Five standard solutions with final concentrations ranging from 0 to 100 μg/mL were
prepared by diluting the stock solution with a methanol/water solution (4/6, v/v). These standard solutions were injected into the HPLC system to calibrate the method.

The melatonin solubility during the extraction process was verified and confirmed by injecting two standards of melatonin freshly prepared from a water-methanol mixture and extracted from Oral Mix SF suspension. Calibration of the method was performed from a freshly prepared pure drug in Oral Mix SF.

Sample Preparation

One hundred microlitres of preparation with a nominal concentration of 2.0 mg/mL were transferred into 1.5 mL centrifuge tubes, and 300 µL of acetonitrile was added for excipient precipitation. The tubes were vigorously mixed using a vortex for 20 s and then centrifuged at 12,000 g for 15 min. Fifty microlitres supernatant was transferred into 1.5 mL centrifuge tubes, and 450 µL methanol/water solution (4/6, v/v) was added. The tubes were vigorously mixed using a vortex for 10 s and transferred to a 96-well plate and then analysed by HPLC.

Forced Degradation Experiments

To validate the stability-indicating capability of the assay, the melatonin standard solution was subjected to extreme ranges of heat and pH. Four separate 1 mg/mL melatonin samples were mixed with 1 mL of the following solutions: water, NaOH (1 mol/L), HCl (1 mol/L) and 3% H2O2. They were then heated to 60°C for 3 h. After cooling down, the solutions were diluted tenfold with a methanol/water solution (4/6, v/v) and assayed by HPLC.

RESULTS

Assay Validation

The accuracy of the analytical method was confirmed by the linearity of the standard curves ($r^2 = 0.9999$) and the quality control samples. Intra-day and inter-day coefficients of variation were between 0.2 and 0.4% and between 1.6 and 3.3%, respectively, for each tested concentration of the calibration curve. The intra-day coefficient of variation was calculated from three replicate injections within the same day, while the inter-day coefficient of variation was calculated from injections performed on three different days.

At time zero, the content uniformity was calculated from six samples. For the preparations made from tablets, the coefficient of variation was 0.86%, and for the preparations made from pure drug, the coefficient of variation was 1.02%.

Forced degradation experiments in the presence of pure water, HCl, H2O2 and NaOH resulted in a melatonin recovery of 101.7, 97.6, 90.2 and 90.0%, respectively. Figure shows representative chromatograms of melatonin standard solution (50 µg/mL; A); melatonin standard solution prepared form pure active using the Oral Mix SF vehicle (50 µg/mL; B); melatonin stressed sample using 1 mol/L NaOH (equivalent to 50 µg/mL prior to degradation; C); and melatonin stressed sample using 3% H2O2 (equivalent to 50 µg/mL prior to degradation; D). These chromatograms confirmed the suitability of the assay to determine the stability of melatonin. The method was able to resolve the vehicle excipients (Figure B) as well as the degradation products of melatonin (Figures C and D).

Stability Study

At each pre-determined time point, preparations were shaken by hand prior to sampling in order to obtain homogeneous suspensions. Organoleptic evaluation as well as pH measurements were performed. No organoleptic changes were observed during the study. All suspensions maintained their initial opaque white color. During the whole study, all pH measurements comprised between 4.47 and 4.66 for preparations made from tablets and 4.44 and 4.55 for preparations made from pure drug.

The stability study was carried out under varied environmental conditions (Tables and ). Neither storage container (syringe and bottle) affected the stability of the recovered initial concentration of the drug. Similarly, both storage temperatures (refrigerated and ambient) showed similar percentages of the melatonin initial concentration recovered. The acceptance criteria (90–110% of the initial concentration) established for stability studies of compounded formulations are then respected for both storage temperatures and containers. Melatonin oral liquids prepared from pure melatonin powder and tablets in the Oral Mix SF vehicle were stable for at least 90 days at room temperature and under refrigeration in both syringes and PET bottle containers.

DISCUSSION

This study evaluated the effect of environmental factors on the stability of oral suspensions prepared with proprietary Oral Mix SF, a dye-free and sugar-free vehicle.
Figure 1 Representative chromatograms of melatonin standard solution, in test samples and under stressed conditions. (A) Melatonin standard solution (50 µg/mL); (B) melatonin standard solution prepared form pure active using the Oral Mix vehicle (50 µg/mL); (C) melatonin stressed sample using 1 mol/L NaOH (equivalent to 50 µg/mL prior degradation); (D) melatonin stressed sample using 3% H2O2 (equivalent to 50 µg/mL prior degradation).

Table 1 Percent of initial concentration of melatonin recovered in suspension after storage in two types of containers under refrigeration using Oral Mix SF vehicle (n = 3)

<table>
<thead>
<tr>
<th>Study day</th>
<th>Pure active ingredient</th>
<th>Commercial drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottle (95% confidence interval)</td>
<td>Syringe (95% confidence interval)</td>
</tr>
<tr>
<td>C&lt;sub&gt;i&lt;/sub&gt; (mg/mL)</td>
<td>2.01 ± 0.03</td>
<td>2.02 ± 0.01</td>
</tr>
<tr>
<td>Day 7</td>
<td>101.6</td>
<td>95.9</td>
</tr>
<tr>
<td>(97.0–106.1)</td>
<td>(95.1–96.8)</td>
<td>(94.3–98.1)</td>
</tr>
<tr>
<td>Day 14</td>
<td>102.0</td>
<td>98.2</td>
</tr>
<tr>
<td>(100.8–103.2)</td>
<td>(97.6–98.9)</td>
<td>(100.0–102.6)</td>
</tr>
<tr>
<td>Day 30</td>
<td>101.3</td>
<td>99.1</td>
</tr>
<tr>
<td>(100.5–102.1)</td>
<td>(96.1–102.0)</td>
<td>(98.4–106.3)</td>
</tr>
<tr>
<td>Day 45</td>
<td>99.3</td>
<td>98.9</td>
</tr>
<tr>
<td>(96.6–102.0)</td>
<td>(98.0–99.9)</td>
<td>(98.9–101.9)</td>
</tr>
<tr>
<td>Day 60</td>
<td>98.2</td>
<td>96.3</td>
</tr>
<tr>
<td>(97.0–99.5)</td>
<td>(95.7–96.9)</td>
<td>(97.5–98.5)</td>
</tr>
<tr>
<td>Day 75</td>
<td>99.4</td>
<td>98.0</td>
</tr>
<tr>
<td>(97.8–100.9)</td>
<td>(96.9–99.2)</td>
<td>(99.1–100.9)</td>
</tr>
<tr>
<td>Day 90</td>
<td>100.6</td>
<td>99.8</td>
</tr>
<tr>
<td>(98.8–102.4)</td>
<td>(99.2–100.4)</td>
<td>(100.1–103.6)</td>
</tr>
</tbody>
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C<sub>i</sub>, Initial concentration.
According to USP standards, the samples in the study were deemed stable if no more than 110% and no less than 90% of the initial drug concentration was present at the time of testing.4 Melatonin oral liquids (2 mg/mL) prepared from both pure drug powder and tablets in Oral Mix SF showed strong stability over 90 days. These findings give the practitioner confidence that the patient’s medication has maintained its strength over time and compounded integrity. Furthermore, they provide pharmacists and practitioners with multiple options for prescription. Extended stability in multiple container types is valuable for situations when materials are on back order.9

The melatonin (2 mg/mL) suspensions can be stored at either room temperature or refrigerated in either an amber plastic (PP) syringe or an amber plastic (PET) bottle. Furthermore, they can be compounded using either bulk drug powder or tablets. The results of this study show equivalent stability to other oral suspending vehicles, demonstrating that this dye-free vehicle can be substituted for compounding when supporting studies are available.7

The study has been successful in producing useful new information regarding the stability of melatonin in a dye-free Oral Mix SF base. Future studies could be conducted whereby dosage, environment and study duration are further varied to provide a larger range of data on the stability of compounded oral suspensions.10

**CONCLUSIONS**

Every year millions of patients use compounded medications.11 In order to deliver the best possible treatment, the compounder requires vital stability material, as provided in this study, to optimise the compounding process.

Compounding pharmacists are required to assign a valid beyond use date for all compounded medication according to USP specifications.4 This study provides experimentally determined stability information for a commonly compounded API: melatonin (2 mg/mL) in oral suspensions using Oral Mix SF. The suspensions were extremely stable in all forms and storage environments. These data are integral to ensure the quality of the extemporaneous oral medications, to optimise patient treatment and to use as a reference tool in pharmacy compounding.

**Competing interests**

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**REFERENCES**

Evaluation of stability of melatonin


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