

# Administration of melatonin mixed with soft food and liquids for children with neurodevelopmental difficulties

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DOI: 10.1111/j.1469-8749.2008.03092.x  
Published online August 27 2008

It is sometimes necessary for the contents of medication capsules to be mixed with certain foods and drinks because children are not always able to swallow the capsules. The compatibility and short-term stability (6h) of melatonin capsules mixed in a variety of liquids and foodstuffs (water, orange juice, semi-skimmed milk, strawberry yogurt, and strawberry jam) were analyzed for degradation. Extraction of melatonin from these common administration vehicles and an analytical assay for the drug and its potential degradation products were developed and validated. The results showed good recovery of melatonin from low- and high-strength capsules for all administration vehicles (between 89% minimum and 111% maximum). The drug was found to be stable in the common liquids and foods tested for up to 6 hours at room temperature (no degradation peak); hence it is unlikely to compromise the results of the Use of Melatonin in Children with Neurodevelopmental Disorders and Impaired Sleep trial.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a natural substance produced by the pineal gland and is responsible for the circadian rhythm producing sleep. It has been recommended for sleep disturbances in children for over 10 years<sup>1</sup> but there is still weak evidence for indications, dosage, and potential adverse effects.<sup>2</sup> Despite this, there has been a dramatic increase in the prescription of melatonin. A recent survey of community and neurodevelopmental paediatricians of 2000 UK children prescribed melatonin found that autism (68%) and attention-deficit-hyperactivity disorder (44%) were the most common associated diagnoses in these children.<sup>3</sup> Other studies have considered the use of melatonin in children with neurological and developmental disorders and/or epilepsy, who have a higher prevalence of sleep disturbances that are frequently chronic and are usually far more difficult to treat than their typically developing peers.<sup>4–9</sup>

Children with autism and neurological impairments present special challenges for drug administration. Children with autism are reported to present with unusual eating habits, feeding difficulties, and restrictive diets.<sup>10</sup> Physical characteristics of food, colour, brand name, or packaging can be important, with any attempt to introduce foods not corresponding to the child's preference being met with refusal.<sup>11,12</sup> Parents often have to be extremely creative either in disguising or mixing medication with the right type of liquid or food.

Many children with learning difficulties and epilepsies are very aware of the different tastes of medication, often sensitized by previous bad experiences with bitter tasting anti-convulsants. Again, parents often go to great lengths to make medicines more palatable. Children with profound neurological disabilities may require gastrostomy tubes for feeding and drug administration. Therefore, all medications require a liquid preparation or dispersion/dissolution in a liquid vehicle before administration.

The present study was part of the preparatory work for the Department of Health, Health Technology Assessment Programme-funded Use of Melatonin in Children with Neurodevelopmental Disorders and Impaired Sleep (MENDS) trial. This is a randomized, double-blind, parallel group trial of melatonin versus placebo in children with a confirmed neurodevelopmental disorder aged 5 years to 15 years 8 months.

Synthetic melatonin is an unlicensed medicine in many countries and is only available in the UK as a 'named patient only' medicine. However, it is classified by the US Food and Drug Administration as a dietary supplement, which is not subject to strict manufacturing quality control and so is of uncertain uniformity of content.<sup>13</sup> For the MENDS trial, immediate-release capsules of pharmaceutical-grade melatonin (strength 0.5, 2, 6, and 12mg) were produced by Penn Pharmaceutical Services Ltd (Tredgar, UK) and supplied by Alliance Pharmaceuticals Ltd (Chippenham, UK).

A previous systematic literature review found that many of the previously published paediatric, randomized controlled trials did not provide adequate information on the formulation and method of administration of the trial drugs, and that the absence of this information severely compromised the reliability and validity of the results.<sup>14</sup> The MENDS study team recognized that many participants of the trial would not be able to swallow capsules. It was considered important,

therefore, to take account of the current pragmatic usage of melatonin, i.e. mixing the contents of the capsule with liquids or foods, to ensure the validity and reliability of the results of the MENDS trial.

In August 2006, investigators from several UK sites conducted a retrospective survey of how parents of children with neurological disabilities, already taking melatonin for sleep disorders, currently administered the drug. The most common vehicles were jam, yoghurt, orange juice, milk, and water. The 'leading' flavour was strawberry for both the jam and the yoghurt.

The aims of this work were to assess the compatibility and short-term stability (over 6h) of the MENDS melatonin capsules (using both low and high doses) mixed in water, orange juice, semi-skimmed milk, strawberry yogurt, and strawberry jam, and to confirm that the method of administration of the melatonin would not impair the targeted therapeutic dose.

## Method

### MATERIAL

Pure melatonin powder (99.5% purity, BN G00140), and the four main melatonin degradation products (methoxy-tryptamine melatonin [BN 507722-4 24], 5-methoxy-3-indolyl acetonitrile melatonin [BN 4060307765-099], diamine melatonin [BN DC/248/048], and diamine acetate melatonin [BN DC/248/051 C]) for preparation of the high-performance liquid chromatography (HPLC) standard were obtained from Sigma Aldrich (Poole, UK).

Melatonin capsules containing 0.5mg (BN 0274B) or 12mg (BN 0287B) of melatonin, lactose, and magnesium stearate in a size 2 white opaque capsule were manufactured by Penn Pharmaceuticals Services Ltd, (Tredgar, UK). Placebo capsules (BN 0198B) were also provided; they contained lactose and magnesium stearate but no melatonin. The filled weight of each capsule was 200mg.

HPLC-grade acetonitrile and methanol were purchased from Fischer Chemicals (Loughborough, UK). Ammonium acetate (BN 1246964) and triethylamine (BN 1302186) were obtained from Fluka (Buchs, Switzerland). Water was deionized in the laboratory by ion exchange using a water purification system from ELGA (High Wycombe, UK).

Orange juice from concentrate, semi-skimmed milk, and strawberry jam were brand products from Tesco Stores Ltd (Cheshunt, UK). The strawberry yogurt was from the Müller-light brand from Müller Dairy (UK) Ltd (Market Drayton, UK).

### ASSAY AND STABILITY-INDICATING HPLC METHOD

A stability-indicating analytical procedure using HPLC was developed and validated for analyzing melatonin and its four main degradation products (methoxy-tryptamine melatonin,

5-methoxy-3-indolyl acetonitrile melatonin, diamine melatonin, and diamine acetate melatonin) in the food products.

We used a Hewlett Packard Series 1050 HPLC system consisting of a gradient pump with a spectrophotometric ultraviolet detector set at a wavelength of 275nm. Chromatographic separation was achieved using a reversed-phase Supelco Discovery HSF5 column (Sigma-Aldrich, Gillingham, UK), 15cm × 4.6mm, 5µm particle size. The column temperature was maintained at 40 °C. The flow rate of the mobile phase was 1mL/min. The mobile phase used for the analysis of melatonin consisted of ammonium acetate (20mM) + triethylamine (43.6mM) adjusted to pH5.0 with glacial acetic acid. Melatonin and its degradation products were separated using gradient elution where the percentage of acetonitrile was gradually increased in the mobile phase (Table I). The injection volume was 20µL and the run time was of 35 minutes per sample analyzed.

Acquisition of chromatograms and calculations (peak detection and integration) were made using PC/Chrom version 4.0.9.0 (H&A Scientific, Inc, Frankfurt am Main, Germany).

The method validation parameters conducted were in accordance with the International Conference on Harmonisation, Technical Requirements for Registration of Pharmaceuticals for Human Use, Text on Validation of Analytical Procedures Q2A. The method was validated for specificity, linearity, precision, accuracy, limit of detection, and limit of quantification.

### EXTRACTION METHODS, COMPATIBILITY, AND SHORT-TERM STABILITY STUDY

The contents of one pharmaceutical placebo capsule (0mg) or low-strength (0.5mg) or high-strength (12mg) capsule were emptied into separate centrifuge tubes and mixed with orange juice (10ml) or water (10ml) using a vortex for 1 minute. The tubes were attached to a vertical mixing wheel (Erweka model number AR 400) and mixed by tumbling (48rpm) for 30 minutes.

For the other three substances, a slightly different method was used. The capsule content was mixed using a homogenizer (Ultra-Turrax, T25 basic from IKA-Werke GmbH & Co. KG, Staufen, Germany; diameter of external blade: 8mm) at 19 000rpm for 1 minute in each vehicle (10ml milk, 5g yoghurt, and 5g jam). For the milk, 10ml of glacial acetic acid was also added to break up the emulsion and to ease subsequent separation and filtration.

After the standing period (0 and 6h at room temperature (20°C [SD1]) unprotected from light), the dissolution solvent (methanol:water, 70:30 by volume) was added up to 50ml and, again, the samples mixed using the homogenizer for a further 2 minutes. All tubes were then centrifuged at 4000 relative centrifugal force for 1 hour (Megafuge 1R Benchtop Centrifuge, Hergeus Instruments, Hanau, Germany). After centrifugation, the supernatants were filtered through a 0.45µm syringe-driven filter unit (Millex MEF 33mm, Millipore, Cork, Ireland) into amber vials and analyzed by HPLC.

A blank sample composed of just the food/beverage undergoing the extraction phase was analyzed at each time point to check that it did not contain any chemicals that could interfere with the chromatogram of melatonin and its degradants. Dispersion of capsules in pure water (10ml) was carried out as a control. All measurements were performed

**Table I: Acetonitrile gradient in mobile phase**

Time (min)	Acetonitrile (%)
0	20
20	Up to 68
25	68
25.1	Down to 20
35	20

in triplicate (with the exception of placebo capsules and blank solutions, which were performed in duplicate).

#### EXPRESSION OF RESULTS

Before each sequence of analysis, two standards of melatonin (exactly approximately 10mg of melatonin in 50ml dissolution solvent) were injected and the analysis was considered reliable if standard comparison was from 97 to 103%. The melatonin sample concentration was calculated against one standard. The amount of melatonin extracted could then be calculated by multiplying the concentration obtained by the dilution factor (50). Finally, the percentage of melatonin extracted was calculated by dividing the amount extracted by the strength of the capsule. The results were expressed as the mean (SD) percentage of melatonin extracted from the capsules.

In parallel, peaks corresponding to the main melatonin degradants were monitored. If any, the total area under the curve (AUC) for those peaks was measured. If significantly greater than the AUC of similar peaks in the corresponding blanks and placebos, it was considered a sign of degradation.

#### EQUIVALENCE OF ADMINISTRATION

For each sample the mean and two-tailed 95% confidence interval (CI) was calculated using the formula  $95\% \text{ CI} = x \pm (t_{95\% \text{ CI}} \times \text{SD}) / \sqrt{n}$ , where  $x$  is the mean amount of melatonin retrieved in each dispersion media (0- and 6-h results pooled), SD is the standard deviation, and  $t$  is 2.571, the value of the  $t$ -distribution statistic for 5 degrees of freedom as  $n=6$  for all samples. This calculation was repeated for each food or beverage tested to provide information about the reliability of the mean and allowing comparison.

Equivalence of administration of both strength capsules in any vehicle would be demonstrated if the 95% CI fell entirely between the largest acceptable dose difference (15% SD of the stated dose) considered therapeutically acceptable. This range was 0.425 to 0.575mg for the 0.5mg capsules and 10.2 to 13.8mg for the 12mg capsules.

## Results

#### OPTIMIZATION AND VALIDATION OF CHROMATOGRAPHIC CONDITIONS

Optimized chromatographic conditions resulted in no co-elution of peaks, and the melatonin peak could be separated clearly from its degradation peaks. Retention times for methoxy-tryptamine, melatonin, 5-methoxy-3-indolyl acetonitrile, diamine, and diamine acetate were 4.2, 7.0, 10.5, 13.1, and 14.1 minutes respectively. This method was therefore considered suitable for quantification of melatonin and its degradation products. Regression analysis (Fig. 1) illustrated linearity of the concentration and peak height over the range 50 to 500µg/ml, with a correlation coefficient ( $r^2$ ) of 0.998 (lower

limit of acceptance 0.995). The results demonstrated an acceptable fit of the data to the regression line as there was a strong correlation between the concentration of the analyte and the AUC.

Precision of injections was required to demonstrate the absence of unacceptable differences between the AUCs for the same concentrations. The relative SD was 0.14% (the acceptable limit being 0.5%), illustrating good precision of the method. The mean percentage recovery of melatonin was 98.3%, which falls within the acceptance criteria of 98 to 102%. This showed that the level of agreement between the true and observed values was accurate. The limit of detection was 24.2ng/ml and limit of quantification was 80ng/ml, which was well below our working concentration of 10 and 240µg/ml for the 0.5 and 12mg capsules respectively.

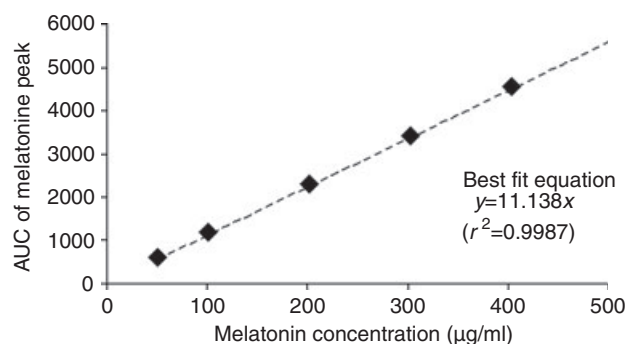
#### COMPATIBILITY AND SHORT-TERM STABILITY OF MELATONIN IN WATER AND FOODSTUFFS

Table II shows a summary of the results of the stability over 6 hours of melatonin in water, orange juice, semi-skimmed milk, strawberry yoghurt, and strawberry jam.

The results show good percentage recovery of melatonin for all food products. The lowest recovery rates were for 0.5mg melatonin, at 6 hours for water (89.4% [SD 0.6]), 6 hours for orange juice (89.3% [SD 4.9]), and 0 hours for yoghurt (89.7% [SD 0.9]). The highest recovery rates were for 0.5mg melatonin, at 0 hours in orange juice (110.9% [SD 14]), and 0 hours for jam (103% [SD 4.2]). Moreover, qualitatively, no degradation peak was observed.

#### DOSE ADMINISTRATION

Plots of the data revealed no gross departures from normality. For all samples the mean was close to the median, and data points were within 3SDs of the mean. As the data were assumed to be normally distributed and the sample size small



**Figure 1:** Calibration curve of melatonin in dissolution solvent (Methanol:water 70:30 v/v). AUC, area under curve.

**Table II:** Mean (SD) percentage of melatonin recovered in different vehicles over 6 hours

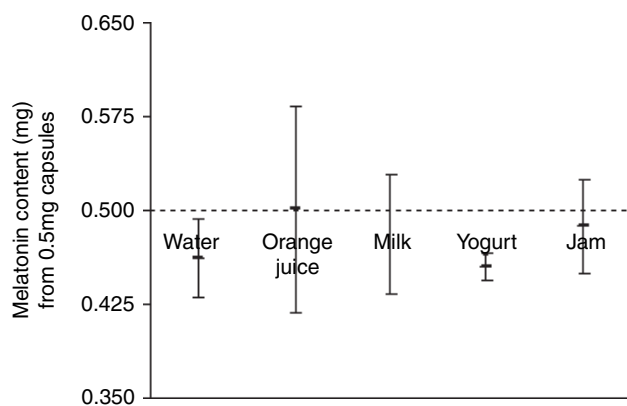
Time (h)	Capsule strength (mg)	Percentage in water	Percentage in orange juice	Percentage in milk	Percentage in strawberry yoghurt	Percentage in strawberry jam
0	0.5	95.1 (7.0)	110.9 (14.0)	98.1 (12.6)	89.7 (0.9)	103.0 (4.2)
0	12	98.6 (0.6)	94.8 (2.3)	95.3 (2.4)	98.9 (1.2)	92.0 (0.8)
6	0.5	89.4 (0.6)	89.3 (4.9)	94.1 (0.9)	92.3 (1.8)	93.4 (6.9)
6	12	98.4 (0.8)	91.3 (3.4)	92.6 (4.8)	97.6 (1.8)	90.1 (7.3)

( $n=6$  after pooling the three repeats at 0 and 6 hours data), the  $t$  distribution could be used to calculate the 95% CI. As illustrated in Figures 2 and 3, all 95% CIs were within 15%SD of the stated dose except for the 0.5mg capsules when mixed with orange juice (95% CI was within 17.5%SD of the stated dose).

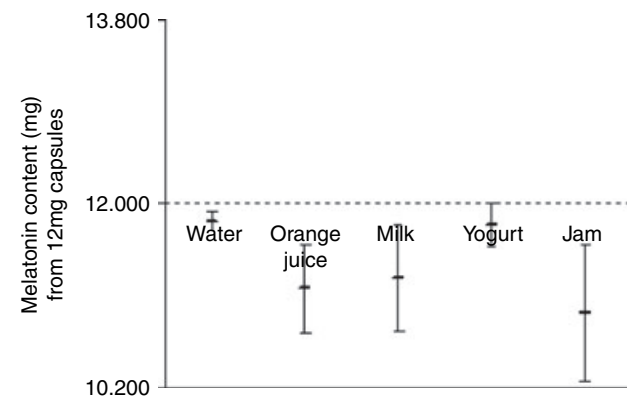
In practice, our findings meant that if the capsules' contents were mixed with one of the five tested vehicles (and even if the mix was kept for up to 6 hours, which is unlikely to happen and not recommended), the patients would receive the therapeutic targeted dose 15%SD, except when the low-dose capsules are mixed with orange juice as it delivered slightly more or less drug: 0.418 to 0.583mg instead of 0.425 to 0.575mg. Nevertheless, this is a minor deviation and was considered clinically acceptable.

### Discussion

Melatonin in synthetic form is being increasingly prescribed to many children with neurodevelopmental disorders and impaired sleep. As young children are unable to swallow these melatonin capsules, they are opened and combined with common foods and liquids. The stability of melatonin in these products over time is, therefore, of concern, and this study was necessary in preparatory work for the MENDS



**Figure 2:** Mean amount of melatonin recovered (95% CI) when low-strength capsules ( $n=6$ ) were mixed in five vehicles.



**Figure 3:** Mean amount of melatonin recovered (95% CI) when high-strength capsules ( $n=6$ ) were mixed in five vehicles.

study. In fact, the broader issues of extemporaneous preparations and other wider difficulties in the use of medicines for children<sup>14-16</sup> reflect the continuous need for this kind of study to be preferred.

### VALIDATION OF THE HPLC METHOD

The test mix chromatogram produced for method specificity showed sharp and symmetrical peaks for melatonin and its four degradation products, with no co-eluting peaks. This enabled us to check easily for any melatonin degradation in the samples. A good linear relation between the concentration of the analyte (50–500 $\mu$ g/ml) and the AUC was confirmed (Fig. 1). Precision of injection results showed very little variation, revealing no error in the functioning of the HPLC machine. Method accuracy was assessed by recovery studies. Quantitative recovery was achieved, validating the accuracy of the method used in the analysis. The lowest concentration of melatonin that could be measured with accuracy and precision (limit of quantification) was found to be 80ng/ml, and our working concentration was well above that value.

### STABILITY OF MELATONIN IN WATER AND FOODSTUFFS

Table II summarizes the results of the compatibility and short-term stability of melatonin 0.5 and 12mg capsules in water, orange juice, milk, strawberry yoghurt, and strawberry jam over 6 hours. A statistical  $t$ -test was used to check if the differences observed at 0 and 6 hours for each food or beverage were statistically significant. The recovery for a 0.5mg melatonin capsule in water and orange juice after 6 hours and in yoghurt at 0 hours yielded the lowest mean results (Table II). The samples for 0.5mg melatonin capsules in orange juice at 0 hours gave the highest percentage (110.9%), with a large SD (15.6%). Nevertheless, all the mean results were within a 100% [SD 11] recovery. We can conclude that there was no significant difference between the samples at 0 hours and those at 6 hours in each medium studied. Moreover, because we observed no degradation peaks, the compatibility and stability of melatonin in these different products could be confirmed.

Note that, to our knowledge, there has been no robust dose range-finding study with melatonin in children. Therefore, the lowest and highest effective doses have never been determined. Nevertheless, for lowest effective dose, there are three studies using low-dose melatonin (0.1–2mg).<sup>5,17,18</sup> In the study by Niederhofer et al.,<sup>18</sup> 0.1mg and 0.3mg doses were effective over an acute period (7 days). Therefore, even if 20% of the 0.5mg dose was retained in the capsule, it could still be predicted to exert a clinical effect. For overages, Jan et al.<sup>19</sup> refer to doses of up to 25mg during the blinded phase of a study, and doses of up to 15mg during an open label follow-on period (mean duration 2y 2mo, mean age at start of open study 8y 7mo). There was only one recorded adverse event (excessive sedation, dose not specified). Therefore, based on the published literature, dose-related adverse events at the high dose are not predicted. In our study, the content of melatonin recovered from the capsules was considered pharmaceutically and clinically acceptable, and safe if within 15%SD of the stated dose: 0.425 to 0.575mg for the 0.5mg capsules, and 10.2 to 13.8mg for the 12mg capsules.

These results suggest that mixing melatonin in common beverages or foods is an acceptable method of drug

administration to children if they are unable to swallow capsules, because the drug remained intact. Stability of the drug for 6 hours provides an additional safety margin, if the parent or carer forgets to give the medication instantly upon mixing the capsules with food or drinks. However, it is expected that the medication would be given straight away after mixing. Nevertheless, there could be differences in bioavailability between those taking melatonin with food and those not, or an effect of the materials of gastrostomy tubes, which should be taken in to account in the results of the MENDS trial.

### Conclusion

In summary, we observed no degradation of melatonin in this study. The mean melatonin recovery was between 89% and 111%. These findings provide initial evidence that melatonin mixed in water, orange juice, semi-skimmed milk, strawberry yoghurt, and strawberry jam is compatible and stable for up to 6 hours. The results appear to confirm that these specific common foods and liquids used to mix and administer the melatonin in the MENDS trial should not compromise the integrity of the drug and should, therefore, ensure delivery of the prescribed dose.

*Accepted for publication 1st April 2008.*

### Acknowledgements

We thank: John Barber, Alliance Pharmaceuticals Ltd, for providing the melatonin capsules and advice in melatonin dosing; Penn Pharmaceutical Services Ltd for advice in melatonin analysis; John Hughes at Biomedical Ltd for help with statistics; and the Health and Technology Assessment for funding the MENDS trial.

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