USAARL Report No. 2000-23

Dose Uniformity of Over-the-Counter Melatonin as Determined by High-Pressure Liquid Chromatography

by
Brian F. Prazinko, Joseph A. Sam, J. Lynn Caldwell and Alfred T. Townsend

Aircrew Health and Performance Division

August 2000

USAARL

U.S. Army Aeromedical Research Laboratory

Notice

Qualified requesters

Qualified requesters may obtain copies from the Defense Technical Information Center (DTIC), Cameron Station, Alexandria, Virginia 22314. Orders will be expedited if placed through the librarian or other person designated to request documents from DTIC.

Change of address

Organizations receiving reports from the U.S. Army Aeromedical Research Laboratory on automatic mailing lists should confirm correct address when corresponding about laboratory reports.

Disposition

Destroy this document when it is no longer needed. Do not return it to the originator.

Disclaimer

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation. Citation of trade names in this report does not constitute an official Department of the Army endorsement or approval of the use of such commercial items.

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified

1b. RESTRICTIVE MARKINGS:

2a. SECURITY CLASSIFICATION:

2b. DECLASSIFICATION / DOWNGRADING:

3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release, distribution unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S): USAARL Report No. 2000-23

5. MONITORING ORGANIZATION REPORT NUMBER(S):

6a. NAME OF PERFORMING ORGANIZATION: U.S. Army Aeromedical Research Laboratory

6b. OFFICE SYMBOL (If): MCMR-UAD

7a. NAME OF MONITORING ORGANIZATION: U.S. Army Medical Research and Materiel Command

6c. ADDRESS (City, State, and ZIP Code): P.O. Box 620577, Fort Rucker, AL 36362-0577

7b. ADDRESS (City, State, and ZIP Code): 504 Scott Street, Frederick, MD 21702-5012

8a. NAME OF FUNDING / SPONSORING ORGANIZATION:

8b. OFFICE SYMBOL (If):

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER:

8c. ADDRESS (City, State, and ZIP Code):

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62787A	30162787A878	OB	360560

11. TITLE (Include Security Classification): (U) Dose uniformity of over-the-counter melatonin as determined by high-pressure liquid chromatography

12. PERSONAL AUTHOR(S): B.F. Prazinko, J.A. Sam, J.L. Caldwell, A.T. Townsend

13a. TYPE OF REPORT	13b. TIME COVERED FROM	TO	14. DATE OF REPORT (Year, Month,	15. PAGE COUNT
Final			2000 August	9

16. SUPPLEMENTAL NOTATION:

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	melatonin, dose uniformity, High-pressure liquid chromatography
06	15		
05	09		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of this study was to determine the dose uniformity among various brands of over-the-counter (OTC) melatonin tablets (or capsules). Melatonin is a substance that may be used by Army aviation personnel, so education in regards to the actual amounts of melatonin contained in each tablet will be beneficial. High-pressure liquid chromatography was used to analyze eight different brands of tablets. From each brand, two lots were selected and six tablets were taken from each lot for analysis. A liquid chromatograph equipped with a C-18 microbore column was used in conjunction with an autosampler to perform two runs per tablet, while also running intermittent standards on a regular basis. Of the brands tested, most were either consistently higher or lower than the amount stated on the label. Some brands showed significant variability. This variability is reason for concern for those users of OTC melatonin. Although such variability may not necessarily cause any side effects, it does indicate laxity in preparation techniques.

20. DISTRIBUTION / AVAILABILITY OF: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area	22c. OFFICE SYMBOL
Chief, Science Support Center	(334) 255-6907	MCMR-UAX-SS

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

Table of contents

Appendix

List of tables

List of figures

Introduction

U.S. Army aviation personnel work at varying times in a 24-hour day. In addition, deployment across various time zones is common, thus requiring personnel to sleep and work at different times than they are accustomed. These changes in sleep/wake cycles lead to problems in obtaining adequate sleep, and thus, performance is compromised due to the lack of adequate rest. Although some people may be able to avoid working during times outside the "normal" alertness period, it is difficult to overcome the problems associated with sleeping outside the normal times.

One strategy that has been used by researchers in the past to help people adjust to working and sleeping outside the usual times is administration of melatonin (Arendt and Deacon, 1997; Dawson, Encel, and Lushington, 1995; Hughes and Badia, 1997; Knauth, 1997; Monk, 1986; Sack, Hughes, Edgar, and Lewy, 1997). While there is controversy over whether melatonin is successful as a hypnotic, there is considerable evidence that it can effectively alter circadian rhythms (Caldwell, 2000; Dawson and Armstrong, 1996; Lewy, Ahmed, Jackson, and Sack, 1992; Zaiden et al., 1994). Despite this, the clinical sleep community is concerned about melatonin's safety. There are many questions concerning melatonin's effects in pregnancy, its interaction with other medications, the impact of its long-term use, etc. (Arendt and Deacon, 1997). Not the least of these concerns is the quality control of over-the-counter (OTC) melatonin preparations (Czeisler and Turek, 1997; Sack, 1996; The National Sleep Foundation, 1997). Since melatonin can be sold as a dietary supplement, the Food and Drug Administration (FDA) has no control over the manufacturing of melatonin and the safety checks for impurities. In addition, the consistency of the stated dose may not be as stringent as those required by the FDA; for example, the actual melatonin content in the tablet compared to the stated dose may vary more than would be allowed by the FDA.

Army aviation personnel are potential users of melatonin, and confidence should be given that what is purchased in the store actually contains the amount of melatonin stated on the label. In order to determine whether OTC melatonin can be assumed to contain the labeled amount of melatonin which it purports, the melatonin made by several manufacturers was analyzed. If OTC melatonin contains the amount of melatonin which is claimed on the label, then it can be used with confidence. If the analyses indicate that some or all of the OTC melatonin preparations are not as the label claims, users can be educated in this regard.

Methods

Materials

Six tablets were analyzed from each of two separate lots of tablets made by eight different manufacturers (see appendix). High-pressure liquid chromatography (HPLC)-grade methanol (Fisher Scientific), melatonin, sodium acetate, ethylenediaminetetraacetic acid (EDTA) disodium salt, and acetic acid (Sigma) were all acquired from chemical supply companies.

Procedure

Two bottles, each from a different lot of melatonin tablets (or capsules), were purchased at various health food and department stores representing eight manufacturers. Six tablets were drawn from each lot and analyzed to determine the melatonin content of each tablet.

An authentic 1 mM (millimolar) melatonin standard stock solution was prepared by dissolving 116.15 mg of N-acetyl-5-methoxytryptamine in 500 mL of HPLC grade methanol. A 3.0 mg/L standard was prepared by dilution of the stock solution with methanol. All standards were stored at 4°C.

Melatonin tablets (all of which were designated by manufacturer's label to contain 3 mg of melatonin) were finely powdered in a non-porous mortar and pestle (gel capsules were opened and the contained powder removed). Melatonin was extracted with 100 mL of methanol (vigorously shaken for 2 minutes). This extract was diluted to a concentration of one tablet per liter. Each sample (1.5 mL) was placed in a microcentrifuge tube (in duplicate), and centrifuged for 5 minutes at 3500 rpm to ensure sedimentation of suspended particles. All samples were stored at 2-8°C.

The procedure used to determine melatonin content in tablets was HPLC, which has been shown in the past to be sensitive to melatonin (Chin, 1990). A Bioanalytical Systems, Inc. (BAS) 200A HPLC instrument with unijet microbore C-18 column and electrochemical detector at 750 mV operated by BAS control software version 1.40 was used in conjunction with a Kontron 460 autosampler. A Neslab circulator maintained a sample temperature of 5°C. The mobile phase was similar to that of Laganà (1995) and consisted of 75% aqueous solution of 100 mM sodium acetate, 50 mM acetic acid, and 0.1 mM disodium EDTA (pH 4.9) and 25% acetonitrile. Using the autosampler, 10 µL injections were made. Melatonin peaks appeared at approximately 7 minutes with a flow rate of 0.05 mL/min at ambient temperature. Column pressure was approximately 1300 psi.

Data analysis

The amount of melatonin per tablet was determined by a comparison of peak areas calculated with BAS report software version 1.4 set for an initial peak slope of 1.000 and a minimum area of 10,000 units. First and second runs for 6 tablets from each lot were injected immediately following a known standard, and peak areas for these 12 runs were compared to the preceding standard peak area. Data were gathered on a total of 16 lots (8 brands - 2 lots each), requiring 3 separate test sessions to complete all runs. For each of the three test sessions, all standards were averaged and analyzed to determine variability of the method. The calculated melatonin content of each tablet was compared with the z-score calculated from the average and standard deviation of all the tablets.

Results

All standards were made to be precisely 3 mg/L. The variability of peak areas resulted in a value of 3.000 ± 0.150 for 12 runs in test session one, 3.000 ± 0.123 for 6 runs in test session two, and 3.000 ± 0.288 for 8 runs in test session three. The overall value for the 26 standard runs was 3.000 ± 0.198 mg/L. The 96 tested tablets had an average value of 3.091 ± 0.460 mg. For 2 of the 96 tablets, only 1 of the 2 runs was completed as a result of equipment synchronization difficulty.

The table shows each brand and their respective tablets' melatonin content averaged from two runs, as well as the tablet's z-score compared to all measured tablets and the lot average (within each brand) with standard deviation for all tablets within that lot. Of the tablets analyzed (across all brands and lots), 12 tablets had z-scores greater than 1 standard deviation above the tablet mean, and 15 tablets had z-scores greater than 1 standard deviation below the tablet mean. One lot within one brand across all 6 tablets had z-scores greater than one standard deviation both above and below the tablet mean, while two lots had no z-scores greater than one standard deviation either above or below the tablet average.

The figure shows average melatonin content for each lot with standard deviation. The solid line represents the overall tablet average and the dotted line represents their standard deviation.

Discussion

This brief study indicates that questions concerning the actual melatonin content of OTC preparations may be justified. When the standard deviation for the tablets is compared to the standard deviation of the procedure standard, it is clear that the 2.3 fold larger standard deviation for the tablets indicates variability in tablet melatonin content which can be attributed to the tablets rather than to the HPLC procedure. While the methods used in this inquiry could result in individual discrepancies, the great difference in variance between the authentic melatonin runs and the tablet runs likely points to differences in actual tablet melatonin content.

These results indicate that users of OTC melatonin should be aware that the variability in doses for individual tablets is high, and they may be taking more or less melatonin than is stated on the package label. Whether this is a health concern or not has yet to be determined. It may not be significant if one consumes 2 mg or 4 mg of melatonin rather than the expected 3 mg. The safety issues of using melatonin have yet to be determined, but it appears from the literature that a small discrepancy in dosage from what is expected may not be a problem. However, further study is needed to determine whether other concerns are warranted about the manufacturing of melatonin. While the dose uniformity of OTC melatonin is a concern, the purity of the tablets (what else may be in the tablet due to manufacturing laxity), as well as other formulation issues that may affect its absorption into the bloodstream should also be studied. Once these variables are taken into account, consumers can be educated about the quality of OTC melatonin.

Table.

Values for means for each brand. Z-scores higher than 1 or lower than –1 are highlighted.

Brand	Lot	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 5	Tablet 6	Mean	SD
1	1	4.183029	3.380429	3.370829	3.026770	3.253036	3.415427	3.438253	0.391593
	Z	2.375668	0.629853	0.608971	-0.13943	0.352747	0.705981		
	2	3.680631	3.781942	3.438091	3.349509	3.021503	3.038436	3.385019	0.316631
	Z	1.282851	1.503224	0.75528	0.562596	-0.15088	-0.11405		
2	1	3.364672	3.327781	3.316267	3.94914	3.303347	3.171938	3.405524	0.274299
	Z	0.595578	0.515333	0.490288	1.866913	0.462185	0.176344		
	2	2.829457	3.330146	3.198975	3.046241	3.568508	2.929076	3.150400	0.272881
	Z	-0.56862	0.520476	0.235155	-0.09707	1.038962	-0.35193		
3	1	2.834334	2.569549	2.757312	2.723407	2.493965	2.648665	2.671205	0.125703
	Z	-0.55801	-1.13397	-0.72555	-0.79930	-1.29838	-0.96188		
	2	4.189923	3.008142	2.802477	3.052682	3.084579	3.326297	3.244016	0.492774
	Z	2.390663	-0.17995	-0.62731	-0.08306	-0.01368	0.512104		
4	1	3.019919	2.681001	2.707624	2.683022	3.097049	3.195714	2.897388	0.233522
	Z	-0.15433	-0.89154	-0.83363	-0.88715	0.013444	0.228061		
	2	3.602439	3.053351	3.277206	3.244723	3.128686	3.104085	3.235082	0.199161
	Z	1.112768	-0.08161	0.405322	0.334666	0.082261	0.028749		
5	1	2.555349	2.600028	2.580022	2.662594	2.758895	2.700966	2.642976	0.078321
	Z	-1.16486	-1.06767	-1.11119	-0.93158	-0.72211	-0.84811		
	2	2.798595	2.570106	2.970367	2.526545	2.946798	3.119878	2.822048	0.235631
	Z	-0.63575	-1.13276	-0.26211	-1.22751	-0.31338	0.063102		
6	1	2.833228	3.290963	3.388253	3.653859	3.384549	3.468144	3.336499	0.275096
	Z	-0.56042	0.435246	0.646872	1.224617	0.638815	0.820651		
	2	3.498522	3.353296	2.805245	3.541155	3.485759	3.269124	3.325517	0.274497
	Z	0.886729	0.570834	-0.62129	0.979465	0.858967	0.387742		
7	1	3.044974	2.496905	2.679193	3.179384	2.291380	2.420076	2.685319	0.356239
	Z	-0.09983	-1.29199	-0.89548	0.192541	-1.73905	-1.45911		
	2	2.433669	2.513502	2.569159	2.937264	2.469229	2.889040	2.635311	0.220459
	Z	-1.42954	-1.25589	-1.13482	-0.33412	-1.35219	-0.43902		
8	1	3.054744	4.298784	2.632921	2.673185	2.726657	2.149340	2.922605	0.734001
	Z	-0.07858	2.627457	-0.99612	-0.90854	-0.79223	-2.04801		
	2	3.321960	3.035843	4.173585	4.250118	3.091534	4.067289	3.656722	0.566538
	Z	0.502671	-0.11969	2.355126	2.521600	0.001448	2.123911		

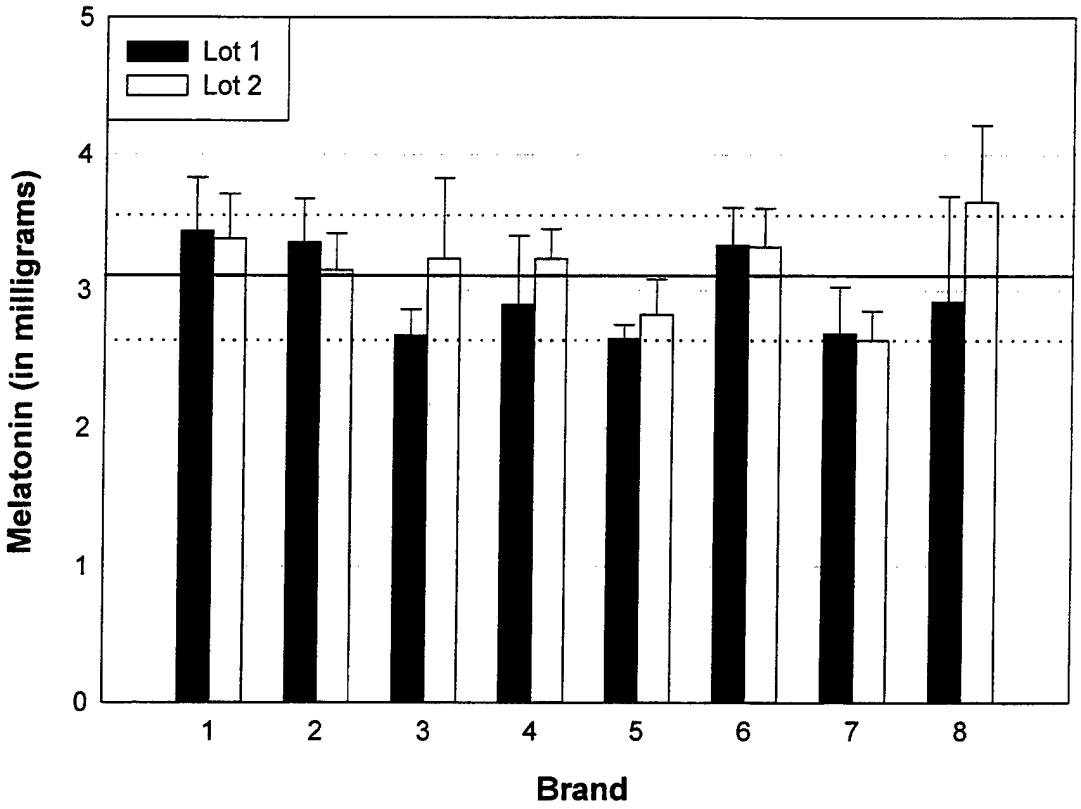

Figure. Means for each brand, respective lot and their standard deviations. The solid line represents the pill grand mean and the dotted lines represent its average standard deviation.

References

Arendt, J., and Deacon, S. 1997. Treatment of circadian rhythm disorders--melatonin. Chronobiology International. 14(2): 185-204.

Caldwell, J.L. 2000. The use of melatonin: an information paper. Aviation, Space, and Environmental Medicine. 71: 238-244.

Chin, J.R.L. 1990. Determination of six indolic compounds, including melatonin, in rat pineal using high-performance liquid chromatography with serial fluorimetric-electrochemical detection. Journal of Chromatography, Biomedical Applications. 598: 111-121.

Czeisler, C.A., and Turek, F. 1997. Is melatonin a treatment for insomnia and jet lag? Washington, DC: National Sleep Foundation.

Dawson, D., and Armstrong, S.M. 1996. Chronobiotics – drugs that shift rhythms. Pharmacology and Therapeutics. 69: 15-36.

Dawson, D., Encel, N., and Lushington, K. 1995. Improving adaptation to simulated night shift: Timed exposure to bright light versus daytime melatonin administration. Sleep. 18: 11-21.

Hughes, R.J., and Badia, P. 1997. Sleep-promoting and hypothermic effects of daytime melatonin administration in humans. Sleep. 20: 124-131.

Knauth, P. 1997. Changing schedules: shiftwork. Chronobiology International. 14: 159-171.

Laganà, A., Marino, A., Fago, G., Pardo-Martinez, B., Bizzarri, M. 1995. Sensitive assay for melatonin in human serum by liquid chromatography. Analytica Chimica Acta. 316: 377-385.

Lewy, A.J., Ahmed, S., Jackson, J.M.L., and Sack, R.L. 1992. Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiology International. 9: 380-392.

Monk, T.H. 1986. Advantages and disadvantages of rapidly rotating shift schedules – a circadian viewpoint. Human Factors. 28: 553-557.

National Sleep Foundation. 1997. Melatonin: The Facts. Washington, DC.

Sack, R. 1996. Advising patients about the use of melatonin. ASDA News. 3: 15-27.

Sack, R.L., Hughes, R.J., Edgar, D.M., and Lewy, A.J. 1997. Sleep-promoting effects of melatonin: at what dose, in whom, under what conditions, and by what mechanisms? Sleep. 20: 908-915.

Zaiden, R., Geoffriau, M., Brun, J., Taillard, J., Bureau, C., Chazot, G., and Claustrat, B. 1994. Melatonin is able to influence its secretion in humans: description of a phase-response curve. Neuroendocrinology. 60: 105-112.

Appendix.

Manufacturers list.

Bioanalytical Systems (BAS)
2701 Kent Avenue
West Lafayette, IN 47906
USA

Fisher Scientific
3970 John's Creek Ct., Ste. 500
Suwanee, GA 30024
USA

General Nutrition Corporation
300 Sixth Avenue
Pittsburgh, PA 15222
USA

IBSD Corporation
P.O. Box 8233
Dothan, AL 36304
USA

Kontron Instruments Limited
Blackmoor Lane
Crowley Business Park
Watford, WD1 8XQ
England

Neslab Instruments
P.O. Box 1178
Portsmouth NH 03802-1178
USA

Nature-Made Nutritional Products
15451 San Fernando Mission Boulevard
Sun Valley, CA 91346
USA

Natrol Incorporated
21411 Prairie Street
Chatsworth, CA 91311
USA

Now Foods
395 South Glen Ellyn Road
Bloomingdale, IL 60108
USA

Nutraceutical International
1400 Kearns Boulevard Floor 2
Park City, UT 84068
USA

Schiff Products, Incorporated
1960 South 4520 West
Salt Lake City, UT 84104
USA

Sigma
P.O. Box 14508
St. Louis, MO 63178
USA

Twin Lab Incorporated
2120 Smithtown Avenue
Ronkonkoma, NY 1779
USA