

Article

Synthetic Melatonin and/or Phytomelatonin Contents in Different Commercial Phytotherapeutic Supplements

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Abstract: In these times and with the pace of life that we have developed, many people need help falling asleep due to poor sleep hygiene, among other reasons. Thus, in mild cases, it is recommended to use natural therapies, such as phytotherapy, avoiding in the first instance the use of drugs. Melatonin is considered a versatile molecule widely used today. It is included as a main ingredient in dietary supplements that are, in some cases, accompanied by medicinal plants as botanical mixes, generating beneficial products for sleep disorders among other conditions. The dietary phytotherapeutic supplements evaluated in this work contain various concentrations of melatonin and other products, resulting in different effects on sleep therapy. The aim of this work is to reveal the quantitative differences that exist between the melatonin contents labeled in the products and those analyzed. The degradation rate of this hormone at three years in the phytotherapeutic supplements is also studied in order to re-evaluate the expiration dates of these products. In conclusion, the mixture between synthetic melatonin and different botanical mixes is very common in the supplements studied here and aimed at improving sleep. However, the most natural thing would be to be able to use only plants with sufficient phytomelatonin content to eliminate the inclusion of chemically synthesized melatonin in preparation. We propose the use of a particular raw plant material with excellent characteristics for this purpose.



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1. Introduction

Melatonin is a widely distributed molecule that was first isolated in 1958 by Dr. Lerner and colleagues from a bovine pineal extract [1]. A year later, in 1959, it was identified in humans [2]. It was not until 1995 that it was isolated in vascular plants [3–5]. Since then, melatonin has been regarded as a pleiotropic molecule due to its various functions, making it a highly relevant and potentially valuable compound [6]. This substance is an indolamine produced and released by the pineal gland in mammals, regulated by a circadian rhythm that peaks during nighttime secretion. In humans, melatonin secretion takes place in the pineal gland. Circadian rhythms are defined as oscillations of biological processes or activities occurring at regular intervals of approximately 24 h [6–9]. Disruptions and dysfunctions of these rhythms can vary in severity, ranging from jet lag, a common sleep disturbance caused by transoceanic travel, to neurological or psychological disorders, including metabolic changes and even obesity. Melatonin influences metabolism by regulating body mass, as it promotes the development of brown adipose tissue while decreasing white adipose tissue accumulation. Consequently, insufficient sleep and the

suppression of melatonin production due to excessive light exposure can lead to metabolic disorders like obesity and type II diabetes [10–15].

It is particularly noteworthy that melatonin is regarded as a powerful scavenger of free radicals, capable of neutralizing as many as ten radicals for each molecule of melatonin [16,17]. It also possesses protective qualities against neurodegenerative conditions, epilepsy, and certain cancers by counteracting oxidative stress. Additionally, numerous studies indicate that melatonin can inhibit inflammatory processes that affect the enzyme cyclooxygenase (COX-2) and enhance the effectiveness of apoptosis in abnormal cells [18–21]. Melatonin has an immunomodulatory effect, enhancing the growth and maturation of natural killer cells, T and B lymphocytes, and monocytes. It also increases antigen presentation in macrophages. Experimental studies have shown that it can inhibit the NLRP3 inflammasome by lowering the infiltration of macrophages and neutrophils in the lungs [22,23]. A recent study proposed that melatonin may be beneficial in managing COVID-19 due to its previously mentioned properties, along with its ability to boost the intracellular enzyme heme oxygenase-1, which has antiviral, anti-inflammatory, antioxidant, and cytoprotective effects and may play a significant role in COVID-19 pathogenesis. Another study suggested that it appears to lower mortality rates in patients infected with the SARS-CoV-2 virus, though the appropriate dosing and treatment regimen have not yet been determined [24–27].

Structurally, melatonin and phytemelatonin are the same molecule. The term melatonin refers to the compound of synthetic or animal origin, while the term phytemelatonin refers to its plant origin [28]. Melatonin in animal cells and phytemelatonin in plant cells have similar biosynthetic routes. Its biosynthesis from tryptophan is common, with the intermediate serotonin and *N*-acetylserotonin [29].

Melatonin consumed globally as a dietary supplement originates from chemical synthesis. Today, the processes for synthesizing melatonin are well established and cost-effective [30–32]. However, in the 1980s, there were notable incidents of poisoning due to improper practices in the chemical synthesis of melatonin and its precursors, which were derived from tryptophan. These practices led to nearly a hundred fatalities and thousands of cases of eosinophilic myalgia syndrome [33–36]. Dietary supplements, including herbal products, are defined by the European Nutraceutical Association (ENA) as “synthetic substances or chemical compounds formulated for specific indications” that differ from pharmaceuticals (drugs) in that they do not have to be specifically approved by regulatory bodies, and a notification accompanied with Certificates of Analysis (CoA) is sufficient.

Figure S1 illustrates three widely used chemical synthesis pathways for melatonin and their byproducts. Scheme A depicts the synthesis of melatonin from tryptophan derivatives, which produces toxic byproducts linked to serious health issues, including eosinophilic myalgia syndrome [30]. In contrast, more recent methods showcased in Scheme B, which involve synthesizing melatonin from phthalimide, raise significant concerns regarding the toxicity of some byproducts produced [31,37]. Furthermore, Fischer’s indole reactions utilizing allylamine, presented in Scheme C, involve hazardous and toxic reagents [38]. In short, both because of the presence of byproducts in synthetic melatonin preparations and also because of the concern of obtaining melatonin of a natural origin, in some cases plant-based dietary supplements with presumed phytemelatonin content have been marketed [32].

In this work, we aim to quantify melatonin/phytemelatonin levels in phytotherapeutic preparations aimed at improving sleep and anxiety. Thus, various commercial preparations containing only synthetic melatonin, containing synthetic melatonin and medicinal-aromatic plants (MAPs), and containing only MAPs were analyzed. The melatonin and/or phytemelatonin contents estimated using a HPLC with fluorimetric detection were assessed and compared with the contents stipulated in phytotherapeutic preparations.

The shelf life of the products was also evaluated according to the amount of melatonin degraded after 3 years.

2. Materials and Methods

2.1. Materials

The samples were obtained by acquiring the products marketed in pharmacies in the “Región of Murcia”, Spain. The melatonin and plant material contents are detailed in Table 1 for each phytopreparation. The data have been collected directly from information on the packaging of the products or the official websites of the laboratories and distributors that market them.

Table 1. List of phytopreparations with their melatonin and plant material contents.

#	Product Trade Name (Lab, Location)	Melatonin Content (in Brochure, mg/pill) (Form)	Content of Plant Material
1	Meladispert Melatonin Herbatonin 100% Vegetal (Vemedica Pharma Lab, The Netherlands)	1.90 mg (tablets)	<i>Oryza sativa</i> <i>Medicago sativa</i> <i>Chlorella vulgaris</i> <i>Chlorella pyrenoidosa</i>
2	Kneipp Sueño Complet (Hartmann Lab, Germany)	1.85 mg (biphasic tablets)	120 mg <i>Valeriana officinalis</i> 80 mg <i>Melissa officinalis</i> 50 mg <i>Passiflora incarnata</i>
3	Farline Melatonin Forte (Farline Lab, Madrid, Spain)	1.85 mg (biphasic tablets)	100 mg <i>Eschscholzia californica</i> 100 mg <i>Passiflora incarnata</i> 50 mg <i>Valeriana officinalis</i>
4	Aquilea Sueño (Uriach Lab, Barcelona, Spain)	1.95 mg (biphasic tablets)	100 mg <i>Eschscholzia californica</i> 100 mg <i>Passiflora incarnata</i> 50 mg <i>Valeriana officinalis</i>
5	PrismaNatural Melato+ (Best Medical Lab, Sevilla, Spain)	1.80 mg (opaque capsules)	117 mg <i>Eschscholzia californica</i> 117 mg <i>Passiflora incarnata</i> 36 mg <i>Melissa officinalis</i> 18 mg <i>Tilia platyphyllos</i> 12 mg <i>Valeriana officinalis</i>
6	Melatonin Zentrum (Ynsadiet Lab, Madrid, Spain)	1.80 mg (transparent capsules)	117 mg <i>Eschscholzia californica</i> 117 mg <i>Passiflora incarnata</i> 36 mg <i>Melissa officinalis</i> 18 mg <i>Tilia platyphyllos</i> 12 mg <i>Valeriana officinalis</i>
7	Dulces Sueños Deliplus (Korott Lab, Alicante, Spain)	1.00 mg (opaque capsules)	200 mg <i>Passiflora incarnata</i> 100 mg <i>Humulus lupulus</i>
8	Arkosueño Forte (Arkopharma Lab, France)	1.90 mg (biphasic tablets)	160 mg <i>Eschscholzia californica</i> 150 mg <i>Valeriana officinalis</i> 100 mg <i>Passiflora incarnata</i>
9	Buenas Noches Total (Eladiet Lab, Barcelona, Spain)	1.85 mg (biphasic tablets)	100 mg <i>Valeriana officinalis</i> 75 mg <i>Passiflora incarnata</i> 25 mg <i>Eschscholzia californica</i>
10	ActiveComplex Melatonin (PharmaNord Lab, Denmark)	1.00 mg (tablets)	-

Table 1. Cont.

#	Product Trade Name (Lab, Location)	Melatonin Content (in Brochure, mg/pill) (Form)	Content of Plant Material
11	Somniplant (Lavigor 7000 Lab, Bizkaia, Spain)	1.99 mg (transparent capsules)	200 mg <i>Passiflora incarnata</i> 100 mg <i>Eschscholzia californica</i> 50 mg <i>Crataegus monogyna</i>
12	Sedador Sueño (PharmaSor Lab, Soria, Spain)	1.80 Mg (film-coated tablets)	150 mg <i>Valeriana officinalis</i> 150 mg <i>Eschscholzia californica</i> 88 mg <i>Passiflora incarnata</i>
13	Dormesan Forte (A. Vogel Lab, Switzerland)	-(liquid)	2090 mg <i>Passiflora incarnata</i> * 1331 mg <i>Melissa officinalis</i> * 1050 mg <i>Avena sativa</i> * 306 mg <i>Valeriana officinalis</i> * 251 mg <i>Humulus lupulus</i> *

* Fresh plant extracts.

2.2. Extraction of Melatonin/Phytomelatonin

One tablet or capsule of each product was triturated with a mortar and homogenized properly. Then, 0.1 g of the sample was weighed on a precision balance in a PVPP tube, and 4 mL of ethyl acetate was added. This process was performed in triplicate for each sample. To extract melatonin/phytomelatonin, the samples were continuously stirred for 15 h in darkness at room temperature (~20 °C), in a rotary stirrer. After, the samples were centrifuged at 7500 rpm for 10 min and the supernatant was decanted to a new tube, thus obtaining the samples free of particles. The supernatant was evaporated under a vacuum using a SpeedVac (ThermoSavant SPD111V, Thermo-Fisher Sci, Waltham, MA, USA) for 4 h at 45 °C. The samples were resuspended in 1 mL of acetonitrile, and ultrasound was applied for 10 min to detach the product from the base of the tube and ensure its dissolution. Also, each tube was stirred by a vortex stirrer for a few seconds to ensure homogeneity and finally filtered with a syringe using 0.22 µm PTFE filters [39].

2.3. Analysis of Melatonin/Phytomelatonin by HPLC with Fluorimetric Detection (LC-FLUO)

The determination and quantification of melatonin/phytomelatonin was performed using a high-performance liquid chromatograph (HPLC). A Jasco model 2000 HPLC was used (Jasco Co., Tokyo, Japan), equipped with an online degasser, quaternary pump, autosampler, thermo-stated column, and a Phenomenex-Luna ODS2 S5 (150 × 4.6 mm) column, coupled to a Jasco FP-2020-Plus fluorescence detector ($\lambda_{\text{excitation}} = 280 \text{ nm}$, $\lambda_{\text{emission}} = 350 \text{ nm}$). The mobile phase, in isocratic form, consisted of a mixture of 82% ultrapure water and 18% methanol, with a flow of 0.3 mL/min at a temperature of 30 °C. The identification of melatonin/phytomelatonin in the samples was carried out by means of the retention times obtained with respect to standard melatonin ($t_R = 12.3 \text{ min}$) and from an in-line fluorescence analysis of the excitation and emission spectra of the molecule using the Jasco ChromNav 2.0 Spectra Manager software [28,39]. Also, melatonin/phytomelatonin identifications using mass spectroscopy analysis with an Agilent LC-chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to 6550 Q-TOF Mass Spectrometer (LC/QTOF-MS) (Agilent Technologies, Santa Clara, CA, USA) were made [39–41].

2.4. Statistical Analysis

Statistical approaches were applied using the SPSS 10 program (SPSS Inc., Chicago, IL, USA), with the LSD multiple range test to establish significant differences at $p < 0.05$. The results are expressed as means with standard error (SE, $n = 4$).

3. Results and Discussion

3.1. Analysis of Melatonin/Phytomelatonin Contents in the Phytotherapeutic Supplements

A peak corresponding to melatonin/phytomelatonin was identified using LC-FLUO (Jasco Co., Tokyo, Japan)) under the specified conditions at approximately 12 min, which matched the retention time of standard melatonin. Additionally, a calibration curve for standard melatonin within the utilized range (0–0.5 ng of injected melatonin) was made [39]. Confirmation of melatonin/phytomelatonin identity in the samples was achieved by adding standard melatonin (at ng levels) to the problem samples, resulting in an observable increase in the suspected peak. Furthermore, validation was obtained through LC/QTOF-MS analysis [42].

LC-FLUO analyses showed excellent sensitivity for the phytotherapeutic samples under study. The results, compiled in Table 2, show that of the 13 phytotherapeutic supplements analyzed, 11 of them show a deficit in their melatonin content between 5% and 67.8%, and only one (#5) of them shows a melatonin surplus of 31.1%, compared to the content announced by the manufacturer.

Table 2. Melatonin estimations in phytotherapeutic products and their quantitative differences respect to announced contents. Mean value $\pm$ standard deviation (n = 3).

#	Product	Advertised Melatonin (mg/pill)	Measured Melatonin (mg/pill)	Difference (mg)	Difference (%)
1	Meladispert Melatonin	1.90 mg	1.47 $\pm$ 0.15	−0.43	−22.6
2	Kneipp Sueño Complet	1.85 mg	1.73 $\pm$ 0.14	−0.12	−6.5
3	Farline Melatonin Forte	1.85 mg	1.17 $\pm$ 0.17	−0.68	−36.8
4	Aquilea Sueño	1.95 mg	1.14 $\pm$ 0.05	−0.81	−41.5
5	PrismaNatural Melato+	1.80 mg	2.38 $\pm$ 0.14	+0.58	+31.1
6	Melatonin Zentrum	1.80 mg	1.56 $\pm$ 0.02	−0.24	−13.3
7	Dulces Sueños Deliplus	1.00 mg	0.95 $\pm$ 0.03	−0.05	−5.0
8	Arkosueño Forte	1.90 mg	1.47 $\pm$ 0.01	−0.43	−22.6
9	Buenas Noches Total	1.85 mg	1.16 $\pm$ 0.12	−0.69	−37.3
10	ActiveComplex Melatonin	1.00 mg	0.58 $\pm$ 0.05	−0.42	−42.0
11	Somniplant	1.99 mg	1.16 $\pm$ 0.11	−0.83	−41.7
12	Sedador Sueño	1.80 mg	0.58 $\pm$ 0.07	−1.22	−67.8
13	Dormesan Forte	-	1.04 μ g $\pm$ 0.35	+1.04 μ g	-

In many cases, the differential between the advertised amount of melatonin and the real amount is significant, being striking with deficits greater than 10% in samples #1, 3, 4, 6, and 8–12, with very high deficits outside the regulations in samples #3, 4, and 9–12 (>30%). Regarding the possible causes of these large differences in the melatonin content of the products, we point out that one of them could be the biphasic nature of the tablets, with a synthetic melatonin content and another herbal component. Although biphasic tablets, which separate the synthetic melatonin layer from that of the herbaceous components, apparently aim for a higher stability and differential bioavailability of both components, this does not seem to be an ideal strategy in terms of their stability. This dual composition could destabilize synthetic melatonin, obtaining much lower valuations than those established during the manufacturing process. Product #5 represents an exceptional case due to its melatonin content surplus being 31.1% of the melatonin content indicated. In this case, the extra content of melatonin does not seem to come from the phytomelatonin content of the herbal component, since other products with the same herbal composition (#6) have a deficit in their differential, so this is assumed to be a problem in manufacturing.

It should be noted that product #5 would not comply with the regulations as it exceeds the limit of 1.9 mg melatonin/tablet for these supplements.

A particular case is product #1, which is presented as a 100% product, that is, without synthetic melatonin, made entirely of original phytemelatonin from the plants and algae that make up its formulation. Our findings indicate that these green algae contain no more than 2–15 ng phytemelatonin/g DW [32], while companion plant species exhibit also very low levels of phytemelatonin, with 1–5 ng/g DW in rice and 16 ng/g DW in alfalfa [43,44]. So, obtaining 500 mg tablets with 1.9 mg phytemelatonin contents seems quite difficult with that composition of herbs and algae. The presence of *Chlorella* suggests that phytemelatonin is primarily obtained by culturing these algae in bioreactors, potentially using precursors like tryptophan, similar to methods used for *Achillea millefolium* [45]. However, there is currently no published information on these phytemelatonin-rich extracts, only their biochemical characterization [46]. Additionally, there is a lack of data regarding the monitoring of cyanotoxin presence in these extracts, which may be contaminated by cyanobacteria (blue-green algae). These cyanotoxins can produce several adverse effects, including carcinogenicity, hepatotoxicity, and neurotoxicity, among others. Consequently, the detection of cyanotoxins in certain algal dietary supplements highlights the urgent need for improved quality control measures [47,48].

Product #13, which does not contain added/specified melatonin, resulted in a content of 1.04 µg of phytemelatonin per tablet. In this case, we would be dealing with a product with full phytemelatonin content (it does not have synthetic melatonin added), and whose herbs provide just one microgram/tablet.

3.2. Study on the Stability of Melatonin in the Phytotherapeutic Supplements

The supplements were first analyzed in 2021, and since then they have been kept under the same conditions until a new measure in 2024. They were kept at room temperature (20–25 °C) and in their original packaging.

Seven of the supplements were tested again in 2024 to assess the amount of melatonin present after this period of time under these conditions. Regarding melatonin degradation after three years, the data indicate a strong degradation that varies from 24.2% to 47.1%, as can be seen in Table 3. Thus, all the data seem to indicate that in addition to possible deviations in the amounts of melatonin formulated (Table 2), the time elapsed since its manufacture seems to be a key factor in terms of the degradation of melatonin in these supplements. In products #4 and #5, the sum of the two percentages (Tables 2 and 3) of deviation with respect to the amount of melatonin indicated exceeds 78%. We cannot draw conclusions about the influence of the type of pill (tablet, capsules, etc.) on melatonin stability. Therefore, it is suggested to shorten the optimal consumption times or, where appropriate, to achieve formulations that manage to stabilize melatonin conveniently in herbal supplements.

Table 3. Analysis of melatonin content in 2021 and 2024. Mean value ± standard deviation (n = 3).

#	Product	2021 Measured Melatonin (mg/pill)	2024 Measured Melatonin (mg/pill)	Difference (mg)	Difference (%)
1	MeladisPERT Melatonin	1.47 ± 0.15	0.89 ± 0.16	−0.58	−39.5
2	Kneipp Sueño Complet	1.73 ± 0.14	1.08 ± 0.08	−0.65	−37.6
3	Farline Melatonin Forte	1.17 ± 0.17	0.96 ± 0.08	−0.21	−17.9
4	Aquilea Sueño	1.14 ± 0.05	0.71 ± 0.09	−0.43	−37.7
5	PrismaNatural Melato+	2.38 ± 0.14	1.26 ± 0.05	−1.12	−47.1
7	Dulces Sueños Deliplus	0.95 ± 0.03	0.72 ± 0.02	−0.23	−24.2
8	Arkosueño Forte	1.47 ± 0.01	0.81 ± 0.02	−0.66	−44.9

4. Conclusions

- In 11 of the supplements, the total melatonin value is below the manufacturer's specifications, between 5 and 68%. It would be advisable to re-examine the products on future occasions to continue checking the veracity of the stipulated contents, but more measures should also be implemented to regulate the amount of actual melatonin they should contain.
- Only in supplements with extra melatonin content (sample #5 with +31.1% and sample #13 with +100%) can we assume that their botanical components truly provide phytomelatonin, which is added as chemical melatonin in all other products.
- Among the proposals to be highlighted, we recommend the performance of residue analyses of the chemical synthesis of melatonin to demonstrate the synthetic origin of this added molecule. We also recommend encouraging the use of natural preparations rich in phytomelatonin that do not contain by-products of the synthetic molecule.
- The stability of the preparations is increased under the following storage conditions: (i) pills in their packaging (blister pack, airtight bottle), (ii) protected from light, and (iii) protected from the action of air (oxidation and changes in color).
- If we consider that the differences in some of the contents measured in 2021 with respect to the manufacturer's specifications are due to early degradation of melatonin molecules, we propose to re-evaluate the established caducity periods by carrying out future studies and analyses.

As a last consideration, the most natural option for dietary supplements containing melatonin would be to only use herbs with sufficient phytomelatonin content to eliminate the inclusion of chemically synthesized melatonin in the preparations. Some recent reviews of the subject can be consulted [32,49], with several of the products developed at the University of Murcia having many possibilities.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nutraceuticals5020009/s1>, Figure S1: Three common routes of melatonin chemical synthesis and their respective byproducts.

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