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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 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 mix, 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. As conclusion, the mixture between synthetic melatonin and different botanical mix 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 the preparations. We propose the use of a particular raw plant material with excellent characteristics for this purpose.

Keywords: dietary supplements; health product; herbs; melatonin; phytomelatonin; phytotherapy; sleep disorder

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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 wasn't 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. The chronobiological variation in the levels of secretion is controlled by α and β noradrenergic receptors. In humans, melatonin secretion takes place in the pineal gland, and both its synthesis and release are regulated by the suprachiasmatic nucleus (SCN) through negative feedback from the hormone itself. The SCN, located in the hypothalamus, is considered the central biological clock of mammals, which also contains peripheral clocks distributed across various organs in the body, with melatonin serving as a marker of circadian rhythms. Circadian rhythms are defined as oscillations of biological processes or activities occurring at regular intervals of approximately 24 hours [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

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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 to 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 [18–20]. This makes it an appealing therapeutic option for inflammatory diseases associated with aging, including cancer [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 proposes 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 suggests 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 phytomelatonin are the same molecule. The term melatonin refers to the compound of synthetic or animal origin, while the term phytomelatonin refers to its plant origin [28]. Melatonin in animal cells and phytomelatonin in plant cells have similar biosynthetic routes. Its biosynthesis from tryptophan is common, with the intermediate serotonin and *N*-acetylserotonin. However, there are some differences at the beginning of the route following (Figure 1) [29].

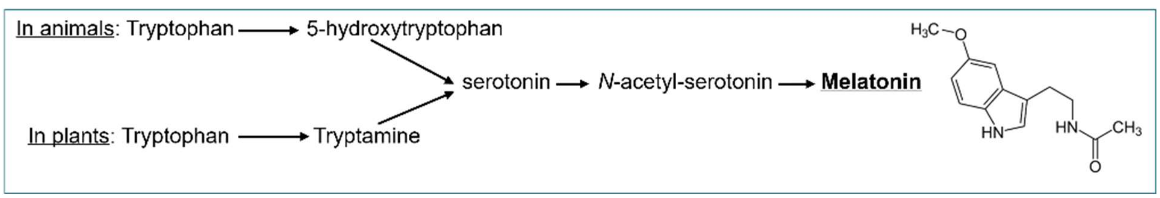

Figure 1. Chemical structure of (phyto)-melatonin or *N*-acetyl-5-methoxytryptamine and the main differences in their biosynthetic pathways.

The 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]. Figure 2 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].

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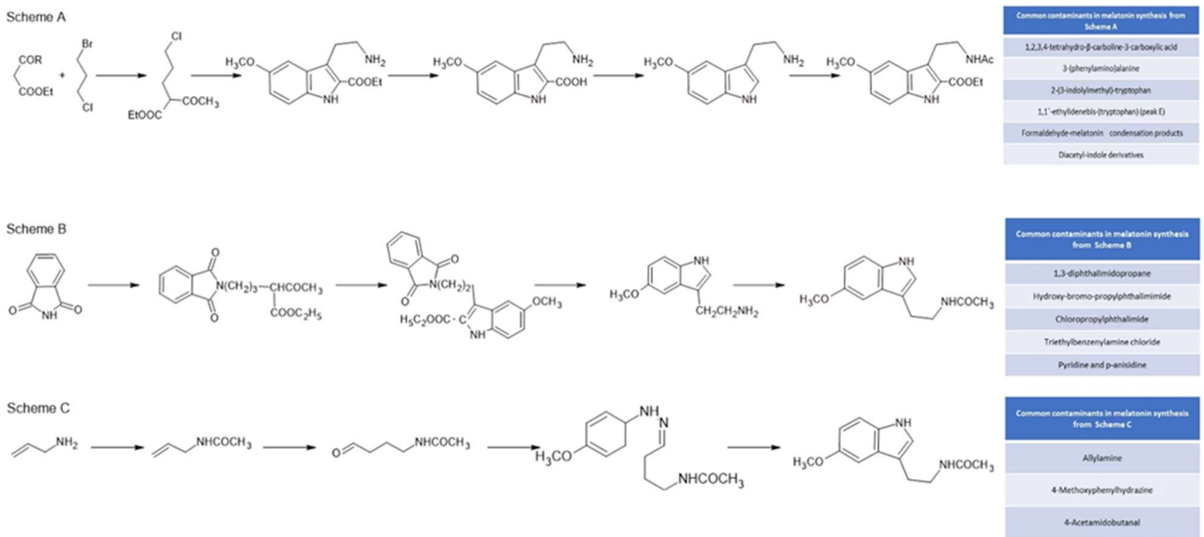

Figure 2. Three common routes of melatonin chemical synthesis and their respective byproducts.

In short, both because of the presence of byproducts in synthetic melatonin preparations and also because of the concern to obtain melatonin of natural origin, in some cases plant-based dietary supplements with presumed phytomelatonin content have been marketed [32].

In this work, we aim to quantify melatonin/phytomelatonin 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 phytomelatonin contents estimated by HPLC with fluorimetric detection were assessed and compared with the contents stipulated in the 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 Region of Murcia (Spain). The melatonin and plant material contents are detailed in Table 1 for each phytopreparation. The data have been collected directly from the information on the packaging of the products or on 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)	**Content of plant material**
1	**Meladispert Melatonin** Herbatonin 100% Vegetal (Vemedia Pharma Lab, Netherlands)	1.90 mg	*Oryza sativa* *Medicago sativa* *Chlorella vulgaris* *Chlorella pyrenoidosa*
2	**Kneipp Sueño Complet** (Hartmann Lab, Germany)	1.85 mg	*120 mg Valeriana officinalis* *80 mg Melissa officinalis* *50 mg Passiflora incarnata*

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3	**Farline Melatonin Forte** (Farline Lab, Madrid, Spain)	1.85 mg	*100 mg Eschscholzia californica* *100 mg Passiflora incarnata* *50 mg Valeriana officinalis*
4	**Aquilea Sueño** (Uriach Lab, Barcelona, Spain)	1.95 mg	*100 mg Eschscholzia californica* *100 mg Passiflora incarnata* *50 mg Valeriana officinalis*
5	**PrismaNatural Melato+** (Best Medical Lab, Sevilla, Spain)	1.80 mg	*117 mg Eschscholzia californica* *117 mg Passiflora incarnata* *36 mg Melissa officinalis* *18 mg Tilia platyphyllos* *12 mg Valeriana officinalis*
6	**Melatonin Zentrum** (Ynsadiet Lab, Madrid, Spain)	1.80 mg	*117 mg Eschscholzia californica* *117 mg Passiflora incarnata* *36 mg Melissa officinalis* *18 mg Tilia platyphyllos* *12 mg Valeriana officinalis*
7	**Dulces Sueños Deliplus** (Korott Lab, Alicante, Spain)	1.00 mg	*200 mg Passiflora incarnata* *100 mg Humulus lupulus*
8	**Arkosueño Forte** (Arkopharma Lab, France)	1.90 mg	*160 mg Eschscholzia californica* *150 mg Valeriana officinalis* *100 mg Passiflora incarnata*
9	**Buenas Noches Total** (Eladiet Lab, Barcelona, Spain)	1.85 mg	*100 mg Valeriana officinalis* *75 mg Pasiflora incarnata* *25 mg Eschscholzia californica*
10	**ActiveComplex Melatonin** (PharmaNord Lab, Denmark)	1.00 mg	-
11	**Somniplant** (Lavigor 7000 Lab, Bizkaia, Spain)	1.99 mg	*200 mg Passiflora incarnata* *100 mg Eschscholzia californica* *50 mg Crataegus monogyna*
12	**Sedasor Sueño** (PharmaSor Lab, Soria, Spain)	1.80 mg	*150 mg Valeriana officinalis* *150 mg Eschscholzia californica* *88 mg Passiflora incarnata*
13	**Dormesan Forte** (A. Vogel Lab, Switzerland)	-	*2090 mg Passiflora incarnata** *1331 mg Melissa officinalis** *1050 mg Avena sativa** *306 mg Valeriana officinalis** *251 mg Humulus lupulus**

**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 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 hours in darkness at room temperature (~20ºC), in a rotary stirrer. After, the samples were centrifuged at 7500 rpm for 10 minutes and the supernatant was decanted to a new tube, thus obtaining the samples free of particles. The supernatant was evaporated under vacuum using a SpeedVac for 4 hours at 45ºC. 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.

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 equipment was used (Jasco Co, Tokyo), equipped with an online degasser, quaternary pump, autosampler, thermo-stated column and a Phenomenex-Luna ODS2 S5 (150 x 4.6 mm) column, coupled to a Jasco FP-2020-Plus fluorescence detector (λexcitation= 280 nm, λemission= 350 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 30ºC. 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= 10.3 min) and from in-line fluorescence analysis of the excitation and emission spectra of the molecule using the Jasco ChromNav 2.0 Spectra Manager software (28). Also, some melatonin/phytomelatonin identification by mass spectroscopy analysis using an Agilent LC-chromatograph (Agilent Technologies) coupled to 6550 Q-TOF Mass Spectrometer (LC/QTOF-MS) were made [39–41].

2.4. Statistical Analysis

Statistical approaches were applied using the SPSS 10 program (SPSS Inc., Chicago, IL, USA), using the LSD multiple range test to establish significant differences at $p < 0.05$. The results are expressed as a 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 under the specified conditions at approximately 12 minutes, 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 [40]. 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.

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, 8-12; and with very high deficits and outside the regulations in samples #3, 4, 9-12 (> 30%). Regarding the possible causes of these large differences in the melatonin content of the products, we could point out that one of them could be the biphasic nature of the tablets, with a synthetic melatonin content and another herbal component. 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 that their melatonin content surplus at 31.1% of the melatonin content announced. 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 herb

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composition (#6) have a deficit in their differential, so it 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 phytomelatonin from the plants and algae that make up its formulation. Our findings indicate that these green algae contain no more than 2-15 ng phytomelatonin/g DW [32], while companion plant species exhibit also very low levels of phytomelatonin, with 1-5 ng/g DW in rice and 16 ng/g DW in alfalfa [43,44]. So, getting 500 mg tablets with 1.9 mg phytomelatonin contents seems quite difficult with that composition of herbs and algae. The presence of *Chlorella* suggests that phytomelatonin 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 phytomelatonin-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].

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

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

#	Product	Advertised melatonin (mg/tablet)	Measured melatonin (mg/tablet)	Difference (mg)	Difference (%)
1	Meladispert Melatonin	1.90 mg	1.47 ± 0.15	-0.43	-22.6
2	Kneipp Sueño Complet	1.85 mg	1.73 ± 0.14	-0.12	-6.5
3	Farline Melatonin Forte	1.85 mg	1.17 ± 0.17	-0.68	-36.8
4	Aquilea Sueño	1.95 mg	1.14 ± 0.05	-0.81	-41.5
5	PrismaNatural Melato+	1.80 mg	2.38 ± 0.14	+0.58	+31.1
6	Melatonin Zentrum	1.80 mg	1.56 ± 0.02	-0,24	-13.3
7	Dulces Sueños Deliplus	1.00 mg	0.95 ± 0.03	-0,05	-5.0
8	Arkosueño Forte	1.90 mg	1.47 ± 0.01	-0.43	-22.6
9	Buenas Noches Total	1.85 mg	1.16 ± 0.12	-0.69	-37.3
10	ActiveComplex Melatonin	1.00 mg	0.58 ± 0.05	-0.42	-42.0
11	Somniplant	1.99 mg	1.16 ± 0.11	-0.83	-41.7
12	Sedasor Sueño	1.80 mg	0.58 ± 0.07	-1.22	-67.8

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13	Dormesan Forte	-	1.04 µg ± 0.35	+1.04 µg	-

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 (20-25ºC) 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 seems 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 the products #4 and #5, The sum of the two percentages (Tables 2 and 3) of deviation with respect to the amount of melatonin announced exceeds 78%. 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/tablet)	2024 Measured melatonin (mg/tablet)	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 5-68%. 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 (+31.1% and +104%) can we assume that their botanical components truly provide phytomelatonin, which is added to the synthetic melatonin added 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).

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- 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 molecule, we propose to reevaluate the established caducity periods by carrying out future studies and analyses.

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

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