

## Original Research Article

# Comparative quality analysis of three marketed melatonin containing products in Spain for the improvement of sleep onset, sleep maintenance and sleep quality.

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### ABSTRACT

**Background:** Exogenous melatonin may effectively improve the sleep-wake cycle and sleep quality in subjects with age or disease related decrease in endogenous melatonin production or response. A controlled release melatonin-based medication, Circadin® has been developed and licensed for insomnia; in addition to a number of food supplements available on the market. However, the quality and release profile of these melatonin containing products may vary.

**Aims:** The aim of the current study was to compare three melatonin containing products, all of them marketed in Spain: a licensed medicine prolonged-release tablet, Circadin® 2 mg, and two food supplements, Aquilea® 1,95mg and Oniria® 1,98 mg.

**Materials and methods:** Three batches of each product were analyzed for melatonin content, inter-batch variability and dissolution profile.

**Results:** Our results confirm a large gap in quality control of the two melatonin containing food supplements, where Aquilea® and Oniria® showed poorer quality control as demonstrated by high variability between batches in melatonin content compared to Circadin®.

**Conclusion:** In terms of the dissolution profile, only Circadin® provides the pro-longed-release profile as indicated in the specifications, with proven efficacy and safety for the improvement of sleep-onset, sleep maintenance and quality throughout the whole night.

*Keywords: Insomnia; Melatonin; Food supplement; Quality control.*

## 1. INTRODUCTION

Insomnia is a subjective complaint on difficulty to fall or stay asleep or poor quality of sleep (nonrestorative sleep) [1].

Melatonin is a naturally occurring hormone produced by the pineal gland and is structurally related to serotonin. Physiologically, melatonin secretion increases soon after the onset of darkness, peaks at 2-4 am and diminishes during the second half of the night.

Melatonin serves as a time cue (signal of darkness) to various organs including the Suprachiasmatic Nucleus (SCN) itself and in the absence of light, may entrain the sleep-wake and neuroendocrine rhythms to the 24 h cycle [2], and enhance sleepiness. Exogenous melatonin may effectively improve the sleep-wake cycle and sleep quality in subjects with age or disease-related decrease in endogenous melatonin production or response [3]. Because melatonin is rapidly metabolized in the liver, a controlled release melatonin-based medication has been developed and licensed for insomnia [4]. In addition, melatonin containing food supplements are available.

There are clear legal differences between medicines (authorized by a Health Authority following submission of an exhaustive dossier including quality, nonclinical and clinical sections demonstrating the efficacy and safety of the product) and dietary supplements. Only medicines must follow the strict quality rule of Good Manufacturing Practice, Good Laboratory Practice and Good Clinical Practice; whereas food supplements do not have such requirements.

In this regard, some papers warn of the lack of quality control of melatonin content and purity in food supplements. In one such studies [5], the authors evaluated 17 melatonin-containing food supplements, identifying tryptophan-related contaminants in 8 of them and, significant deviations from melatonin content declared on the label (from -60% to -20%). In another study in Canada, analyzing 30 melatonin containing food supplements, the variability in melatonin content was found to be -83% to +478%, which is much greater than the 10% margin of the label claim [6]. In the same study, 26% of the supplements were also found to contain contaminants such as serotonin [7] in a CDC report confirm these data and highlight the importance of the risk of serotonin toxicity in children.

The aim of the present study was to compare the pharmaceutical quality between a melatonin-containing medicine and two food supplements available in Spain.

## **2. MATERIAL AND METHODS**

### **2.1. Materials and reagents**

The study compares three melatonin containing products, all of them marketed in Spain: a prolonged-release tablet (Circadin® 2 mg) [8], licensed medicine; a bilayer tablet (Aquilea®) [9], 1,95 mg (first layer of immediate-release melatonin; second layer of valerian, passiflora and California Poppy - Aquilea®); and a prolonged-release film-coated tablet (Oniria® 1,98 mg) [10] (1mg immediate release; 0,98mg prolonged-release, Oniria®). Three batches of each product were analyzed.

All reagents used were of analytical-reagent grade: Melatonin (99.5%) was purchased from Merck (Spain), melatonin related compound A was purchased from USP (Rockville, USA). Monobasic potassium phosphate (Scharlab), orthophosphoric acid 85% (Scharlab), hydrochloric acid 35% (Scharlab) and ammonium acetate (Panreac). Methanol supra-gradient grade, acetonitrile supragradient grade and ultra-pure water (water purification system Milli-Q® IQ 7000 from Merck), were used for chromatographic assays.

### **2.2. Quality attributes: Melatonin Content**

Melatonin content was determined by High-Performance Liquid Chromatography (HPLC) method indicated in the Melatonin Tablets monograph (2019) from the United States Pharmacopeia.

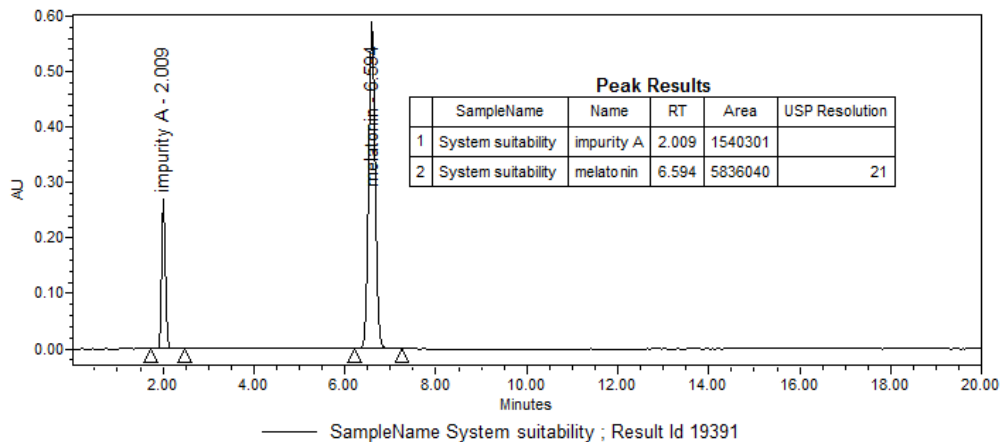
Chromatographic methods were performed using two HPLC systems: System 1 consisting of an isocratic HPLC pump (Waters 1515), an autosampler injector (Waters 717 plus), an in-line degasser (Waters AF Model Code DG2), a column heater (Waters 1500 CHM) and an UV-Visible detector (Waters UV-Visible 2489). System 2 consisting of a binary HPLC pump (Waters 1525), an autosampler injector (Waters 717 plus), an in-line degasser (Waters ILD), a column heater (Waters 1500 CHM) and a PDA detector (Waters PDA 2996). Empower® 3 software (Feature Release 4) was used for instrument control, data acquisition and data analysis.

About chromatographic conditions, mobile phase composition was phosphate buffer pH= 3.50 (filtered by 0.45 µm nylon) and ultragradient grade acetonitrile 75:25 (v/v). Analysis was carried out with a flow rate of 1 mL/mL. Samples were analysed using an Injection volume of 10 µL with a run time of 20 minutes, the UV detection wavelength used was 222 nm. Separation was performed on a Kromasil ODS column (4.6 mm x 150 mm, 5 µm) at 25°C. Samples from different formulations were prepared by taking 20 tablets at random and powdering them in a mortar; the amount corresponding to the dose was dissolved in 20 mL of mobile phase sonicating in an ultrasonic bath for at least 5 minutes, samples were filtered using by 0.45 µm PVDF syringe filters. Content of melatonin was expressed as the percentage of labelled amount of melatonin in the formulations.

As an official method was used, the directed system suitability test was applied in each of the content analysis and suitability requirements stated in the monograph were checked.

A resolution solution (containing USP melatonin RS 0.1 mg/mL and USP melatonin related compound A RS 0.02 mg/mL) was injected at the beginning of each analysis and resolution between peaks was checked. Established specification

(resolution no less than 4) was met for all the analysis. Representative chromatogram for resolution solution is shown below (Figure 1).



**Figure 1. Representative chromatogram of the resolution solution for melatonin content assay.**

Apart from the resolution requirement, the official monograph states that the relative standard deviation for a standard solution (containing USP melatonin RS 0.1 mg/mL) must be less than 2.0%. To quantify samples a five-fold injection of a standard solution was injected; the coefficient of variation for melatonin peak areas was calculated; the established specification was met in all the melatonin content analyses carried out.

### 2.3. Quality attributes: Hardness

Hardness was tested for the formulations tablets using a Sotax Tablet Hardness tester MT50-easy; one tablet at a time was placed into the tester and the force (N) to break the tablet was measured (n = 10). Loss on drying was determined by a gravimetric method using a Sartorius Infrared Moisture Analyzer MA35; an assay was carried out using between 1 and 3 g of the pulverized tablets; results were expressed as a percentage of weight loss (w/w).

### 2.4. Dissolution profiles

Dissolution profiles were conducted in a PhEur, USP type 2 apparatus with 12 vessels (708-DS Dissolution Apparatus, Agilent). Two dissolution media were used, distilled water (500 mL) as in USP and Hydrochloric acid 0.1 N (900 mL) as previously described [11].

Chromatographic methods were performed using the same two HPLC systems as mentioned in Section 2.2: System 1 consisting of an isocratic HPLC pump (Waters 1515), an autosampler injector (Waters 717 plus), an in-line degasser (Waters AF Model Code DG2), a column heater (Waters 1500 CHM) and an UV-Visible detector (Waters UV-Visible 2489). System 2 consisting of a binary HPLC pump (Waters 1525), an autosampler injector (Waters 717 plus), an in-line degasser (Waters ILD), a column heater (Waters 1500 CHM) and a PDA detector (Waters PDA 2996). Empower® 3 software (Feature Release 4) was used for instrument control, data acquisition and data analysis.

Tablets were exactly weighed on an analytical scale and each one was placed in a glass of the dissolution bath at 37°C temperature and 50 r.p.m stirring speed. At the corresponding times (3, 5, 10, 15, 30, 45, 60, 120, 180, 240, 300, 360, 420 and 480 min), 5 mL were taken from each through a cannula with a full flow filter 45 µm, volume was re-placed with dissolution medium. Samples were filtered by 0.45 µm PVDF syringe filters to inject into the chromatographic system.

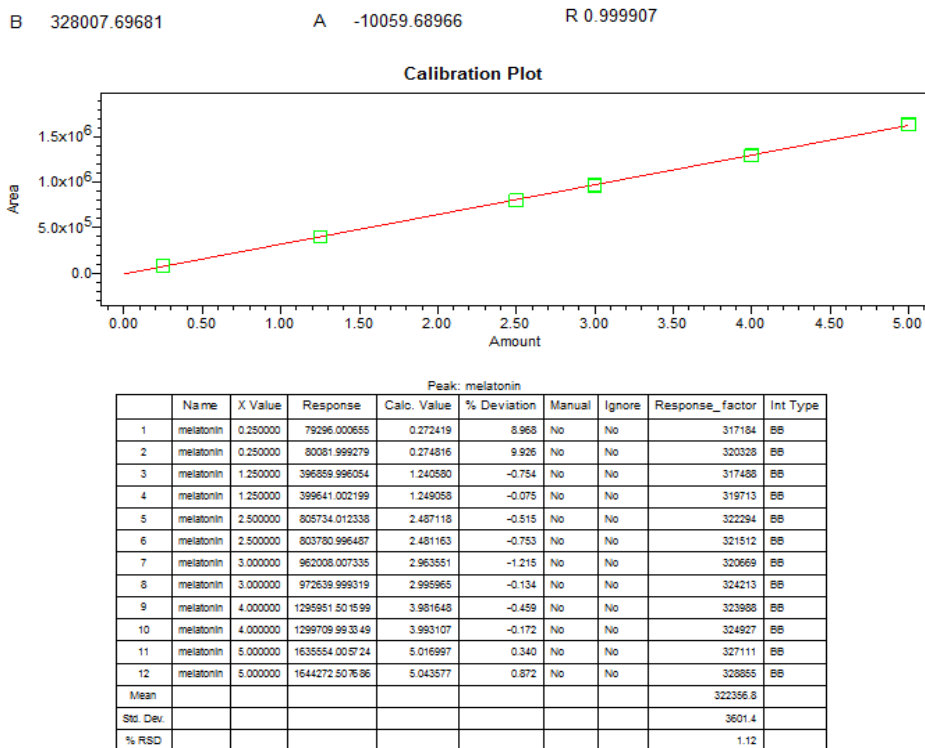
Chromatographic mobile phase composition was ammonium acetate solution 0.6%(w/v) and ultragradient grade methanol 25:75 (v/v). Analysis was carried out with a flow rate of 0.5 mL/mL. Samples were analysed using an Injection volume of 50 µL with a run time of 7 minutes, the UV detection wavelength used was 228 nm. Separation was performed on a Luna C18 column (4.6 mm x 150 mm, 3 µm; Phenomenex) at 40°C.

For USP conditions (500 mL), from a stock solution of melatonin in mobile phase 0.1 mg/mL a serial dilution was performed using a dissolution medium as a solvent to obtain a series of melatonin standard solutions (0.25, 1.25, 2.5, 3.0, 4.0 and 5. µg/mL) for construction of the calibration curve used. In addition, for No USP conditions (900 mL), from a stock

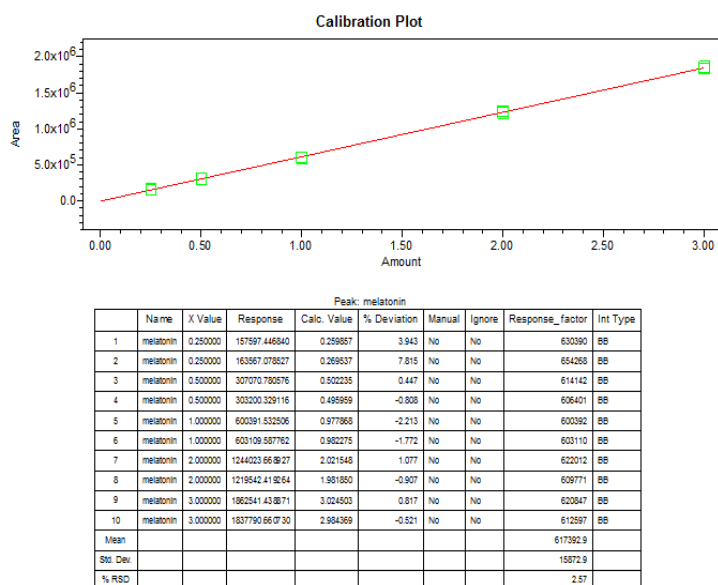
solution of melatonin in methanol 25.0 µg/mL a serial dilution was performed using dissolution medium (Hydrochloric acid 0.1N) as a solvent to obtain a series of melatonin standard solutions (0.25, 0.50, 1.0, 2.0, and 3.0 µg/mL) for construction of the calibration curve, used.

In order to validate the values of the percentage of dissolution obtained in the profiles, the following acceptance criteria were established: the correlation coefficient (R) must be at least 0.990 and the relative standard deviation of the response factors (melatonin peak area divided by the theoretical concentration) is less than 5.0%.

Stablished specifications were met for all the analysis. Representative calibration curve for each of the dissolution conditions are shown below (Figure 2 and Figure 3).



**Figure 2. Representative calibration curve for dissolution test under USP conditions (500 mL water)**



**Figure 3. Representative calibration curve for dissolution test under NO USP conditions (900 mL hydrochloric acid 0.1N)**

### 3. RESULTS AND DISCUSSION

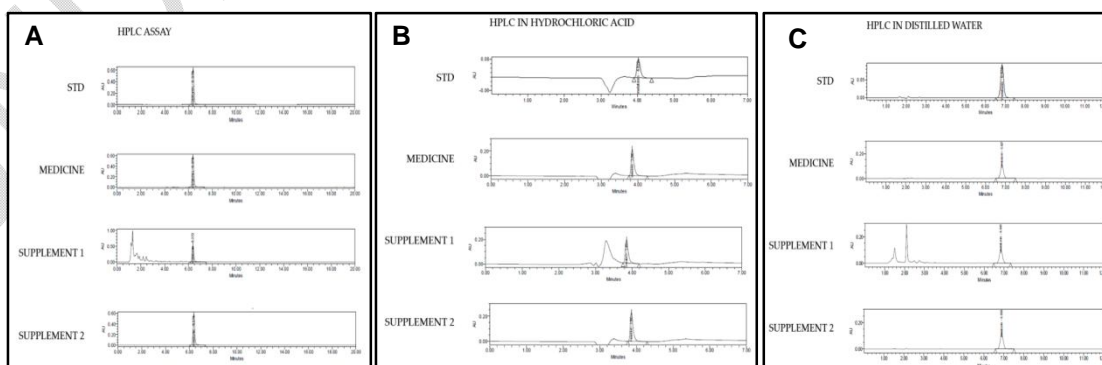
#### Quality attributes

The results of quality attributes analyzed in three batches of each product are summarized in Table 1. As to content, the food supplement Aquilea® showed a melatonin content significantly lower than the labelled amount and higher variability between batches (Table 1); whereas Oniria® and Circadin® had a high percentage of melatonin content with little variability. In terms of purity, as it is shown in the chromatograms in Figure 4, Supplement 1 (Aquilea®), presented several peaks of the unknown source which are not seen in the standard, nor in Circadin®, and could represent unidentified impurities or decomposition products. As for Oniria® some impurities can be observed, especially in (B) HCl medium (Figure 4).

**Table 1. Quality attributes.**

Test	Circadin®	Aquilea®	Oniria®
Content (% of labelled)	97.3[0%]	74.6[18%]	99.1[1%]
Weight of tablet (mg)	169.2[0%]	667.2[32%]	130.0[1%]
Hardness (N)	69.3[8%]	213.0[23%]	56.3[7%]

*All results are expressed as Mean [Coefficient of variation]*



**Figure 4. Representative chromatograms of the standard, Medicine (Circadin®), Supplement 1 Aquilea®) and Supplement 2 (Oniria®) in (A) HPLC assay conditions, (B) HPLC in HCl medium and (C) HPLC in distilled water.**

Dissolution profiles showed a higher variability between batches in the supplements whereas the Medicine had a very consistent profile in either of the two-dissolution media assayed (Figure 5).

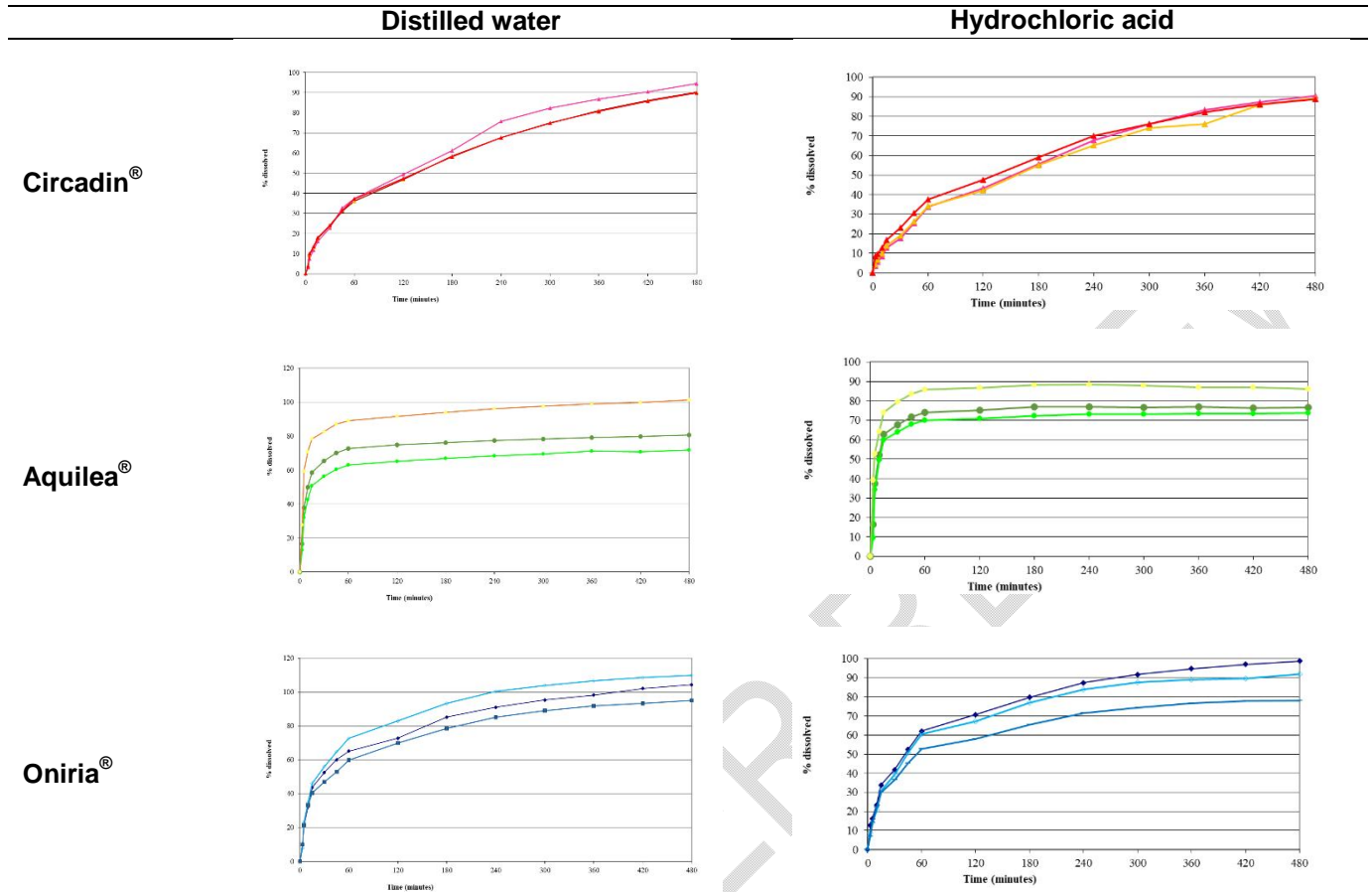


Figure 5. Dissolution profiles of three batches of each product, with distilled water medium (USP) and hydrochloric acid medium.

The medicine Circadin® showed a typical prolonged release profile, showing a release about 70% at time 4h, followed by a sustained release up to 8h. Aquilea® supplement showed a characteristic immediate release profile, considering a melatonin release >60% during the first hour, between X1-Y1% of content at time 4h levelling off at X2-Y2 % at time 8h. Finally, Oniria® supplement showed an intermediate profile, considering a melatonin release >60% at 1h, and X3-X4 was released by 4h. There was a remarkable batch to batch variability in both Supplements, whereas the medicine showed a more reproducible profile.

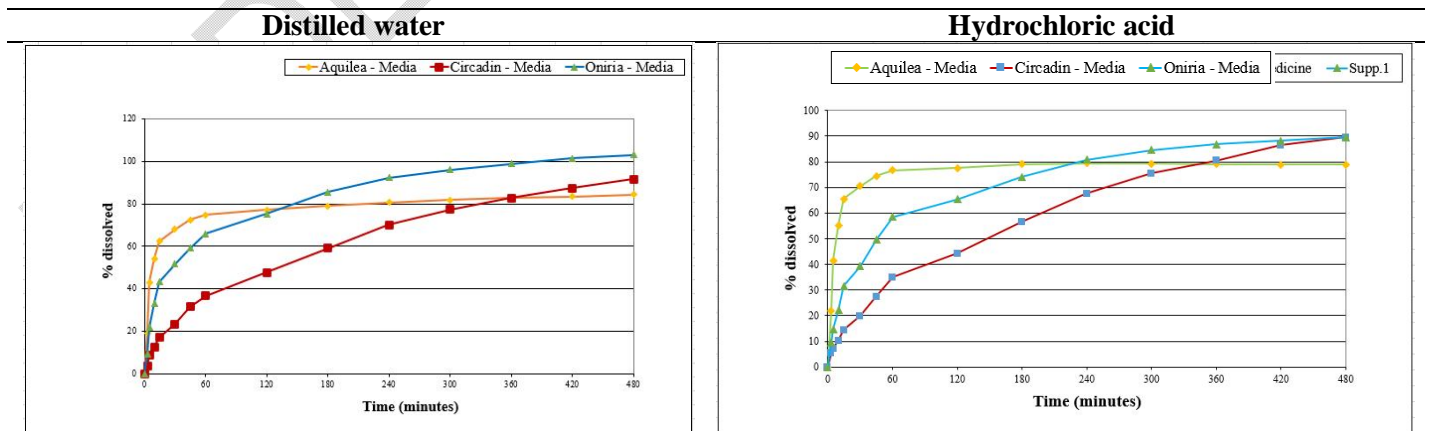


Figure 6. Mean dissolution profiles of three batches of each product, shown in Figure 2, with distilled water medium (USP) and hydrochloric acid 0.1N medium in % of the labelled content.

It is well known that the regulatory pathway, is very strict in the case of medications; they have to stand in specifications of the drug product (active ingredient content and purity, specified release profile, stability under storage conditions) indications of use (in this case, to treat insomnia in subjects 55 years and older) dose (2mg) and safety follow up (including the support of a drug-safety or pharmacovigilance system) and the supply is under prescription. No such requirements exist for food supplements, mostly used to alleviate the symptoms of jet lag and are free on sale as food supplements in pharmacies Internet or other sources without control on quality, dosing or safety (not covered by a pharmacovigilance system). The widening use of melatonin food supplements has called the attention of the Centers for Disease Control and Prevention [7] to warn against products with poor formulation or poor-quality control [12]. Hence, some publication alert that food supplements can have a melatonin content significantly lower than the labelled [5] or large variability between lots [13] or impurities which may some cases be harmful.

However, due to specific regulatory differences between medicines and dietary supplements in development, manufacturing, and marketing, in some cases, the lack of any quality control has affected the quality of melatonin containing food supplements [14]. Therefore, the main objective of the present study was to compare the pharmaceutical quality between a melatonin-containing medicine and two food supplements, all of them with a similar pharmaceutical form, in terms of specific parameters such as melatonin content, tablet weight, hardness, purity and the amount of excipients, as well as in terms of the dissolution profile of each melatonin tablet.

Our results confirm a large gap in quality control of the two melatonin-containing food supplements compared to the licensed medicine (Circadin®). We demonstrated several irregularities such as significant batch-to-batch variabilities in melatonin content, unknown impurities, and a non-specified release profile to the level that can ascertain efficacy and safety, presence of suspected impurities with potential hazards and risks. Importantly, the food supplements, and particularly Aquilea®, presented higher variability between batches, which could be attributed to less exhaustive quality controls in the production process. In addition, Aquilea® showed a significantly lower melatonin content than the labelled value, as well as more impurities, to a level which can seriously compromise the expected effect. These results are consistent with previous studies evaluating melatonin containing food supplements, in which four out of seventeen supplements showed significant deviations from the melatonin content declared on the label [5]. Overall, these results revealed higher homogeneity for the melatonin medicine, which would be indicative of more strict quality controls during the elaboration process. Oniria® presented lower intra-batch variability in content than Aquilea®, but had a high variability in the release profile, which is important for efficacy and safety of the product and furthermore, presented unknown peaks in the analysis that might represent impurities or decomposition products with unknown hazards. The presence of impurities with unknown health risks in melatonin food supplements has also been documented in a CDC report [7].

The release profile of melatonin medication constitutes another key aspect with the aim to improve the sleep quality, sleep-onset or sleep-maintenance difficulties in insomnia. As previously discussed, melatonin is rapidly absorbed and presents a short half-life. Therefore, maintaining effective melatonin concentrations during the night requires either high doses of immediate-release forms or, preferably, a prolonged-release formulation [15]. Among the three melatonin formulations studied in the present work, only the medicine showed a prolonged-release profile in both in vitro dissolution medias tested. Specifically, the melatonin medication presented an initial faster release followed by a slow prolonged release up to eight hours, which is suitable for promoting the induction and maintenance of sleep [11]. Contrary to the medication, Aquilea® reached the asymptote within the first hour of the study, corresponding to an immediate release profile, and Oniria® presented an intermediate release profile but did not maintain the sustained release for 8 hours as required in a prolonged-release profile. In addition, again the medicine revealed the lowest variability between batches, which is in accordance with the previously evaluated parameters and would indicate the implementation of quality controls required by the regulatory agencies that do not exist with food supplements. It should also be noted that the release profile of food complements has never been tested or shown to be effective and safe in any indication and in particular insomnia patients.

Taken together, these results demonstrate that the melatonin containing medication Circadin® exhibits suitable parameters for the treatment of insomnia, presenting high homogeneity between batches, appropriate melatonin content in accordance with the content declared on the label, no impurities, and a sustained release of effective melatonin concentrations throughout the night which is required for sleep maintenance and good quality of sleep [16]. These findings highlight the importance of the exhaustive quality and safety controls required for medications by the regulatory agencies, which are not required for dietary supplements. Quality control issues should prompt a health legislation intervention in quality and safety control of food supplements.

#### **4. CONCLUSION**

The analyzed melatonin containing food supplements, Aquilea® and Oniria®, show poorer quality control as demonstrated by high variability between batches in melatonin content, release profile, and impurities of unknown origin.

The study confirmed the high quality of the medicinal product Circadin® with low variability between batches, content, release profile and purity that is within specifications due to the strict quality control according to GMP. Last but not least, considering the dissolution profile, only Circadin® provides the prolonged-release profile as indicated in the specifications, with proven efficacy and safety for the improvement of sleep-onset, sleep maintenance and quality throughout the whole night.

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