Comparative quality analysis of three marketed melatonin containing products in Spain for the improvement of sleep

.

.

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 several food supplements available on the market. However, the quality and release profile of these melatonin containing products may vary.
Aims: The current study aimed 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®. Moreover, the extended release characteristics of Circadin® were evident in its profile, which exhibited an initial release of 70% at the 4-hour mark, followed by a consistent and sustained release lasting up to 8 hours.
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 of difficulty falling or staying 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 Supraquiasmatic Nucleus (SCN) itself and in the absence of light, may entrain thesleep–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 study [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 that 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 confirms these data and highlights the importance of the risk of serotonin toxicity in children.

The present study aimed 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; a 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 the United States Pharmacopeia (USP) (Rockville, USA) [11]. 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 a UV-Visible detector (Waters UV-Visible 2489). System 2 consists 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, the 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 25ºC. Samples from different formulations were prepared by taking 20 tales 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 0.45 µm PVDF syringe filters. The content of melatonin was expressed as the percentage of the 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 analyses and the 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. The stablished specification

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

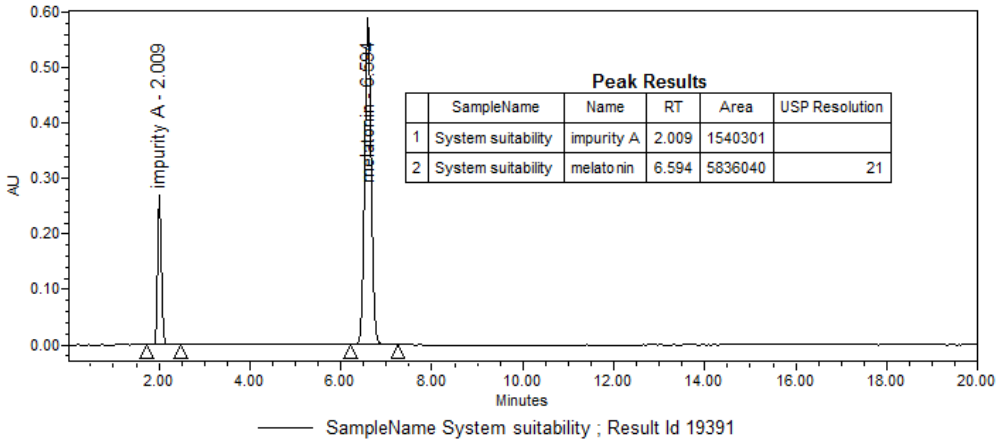


Figure 1. Representative chromatogram of the resolution solution for melatonin content assay.

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 formulation 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, Agillent). Two dissolution media were used, distilled water (500 mL) as in USP and Hydrochloric acid 0.1 N (900 mL) as previously described [12].

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 a UV-Visible detector (Waters UV-Visible 2489). System 2 consists 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 37ºC 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.

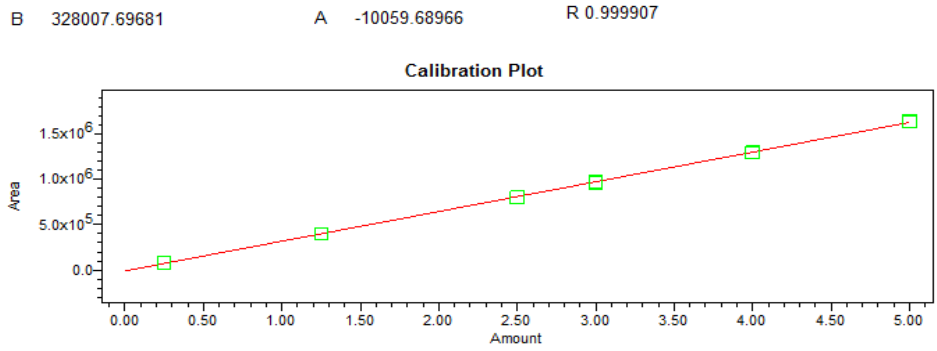
Chromatographic mobile phase composition was ammonium acetate solution 0.6%(w/v) and ultragradient grade methanol 25:75 (v/v). Analysis was carried out with a flow rate of 0.5 mL/mL. Samples were analysed using an Injection volume of 50 µL with a run time of 7 minutes, the UV detection wavelength used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

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.

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%.

Established specifications were met for all the analyses. Representative calibration curves for each of the dissolution conditions are shown below (Figure 2 and Figure 3).

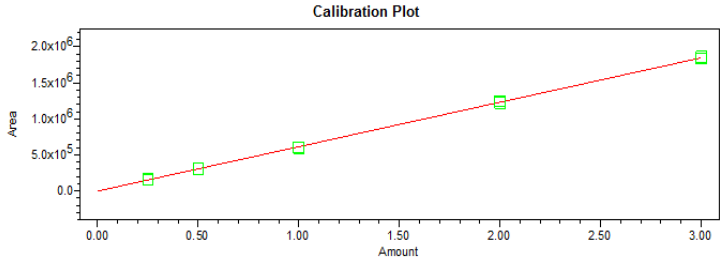


Peak: melatonin

	Name	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	Response_factor	Int Type
1	melatonin	0.250000	79296.000655	0.272419	8.968	No	No	317184	BB
2	melatonin	0.250000	80081.999279	0.274816	9.926	No	No	320328	BB
3	melatonin	1.250000	396859.996054	1.240580	-0.754	No	No	317488	BB
4	melatonin	1.250000	399641.002199	1.249058	-0.075	No	No	319713	BB
5	melatonin	2.500000	805734.012338	2.487118	-0.515	No	No	322294	BB
6	melatonin	2.500000	803780.996487	2.481163	-0.753	No	No	321512	BB
7	melatonin	3.000000	962008.007335	2.963551	-1.215	No	No	320669	BB
8	melatonin	3.000000	972639.999319	2.995965	-0.134	No	No	324213	BB
9	melatonin	4.000000	1295951.501599	3.981648	-0.459	No	No	323988	BB
10	melatonin	4.000000	1299709.993349	3.993107	-0.172	No	No	324927	BB
11	melatonin	5.000000	1635554.005724	5.016997	0.340	No	No	327111	BB
12	melatonin	5.000000	1644272.507586	5.043577	0.872	No	No	328855	BB
Mean								322356.8	
Std. Dev.								3601.4	
% RSD								1.12	

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

B 616695.10658 A -2654.89957 R 0.999859



Peak: melatonin

	Name	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	Response_factor	Int Type
1	melatonin	0.250000	157597.446840	0.259857	3.943	No	No	630390	BB
2	melatonin	0.250000	163667.078627	0.269537	7.815	No	No	654268	BB
3	melatonin	0.500000	307070.780576	0.502235	0.447	No	No	614142	BB
4	melatonin	0.500000	303200.329116	0.495959	-0.808	No	No	606401	BB
5	melatonin	1.000000	600391.532506	0.977868	-2.213	No	No	600392	BB
6	melatonin	1.000000	603109.557762	0.982275	-1.772	No	No	603110	BB
7	melatonin	2.000000	1244023.663927	2.021548	1.077	No	No	622012	BB
8	melatonin	2.000000	1219542.419264	1.981850	-0.907	No	No	609771	BB
9	melatonin	3.000000	1862541.438871	3.024503	0.817	No	No	620847	BB
10	melatonin	3.000000	1837790.660730	2.984369	-0.521	No	No	612597	BB
Mean								617392.9	
Std. Dev.								15872.9	
% RSD								2.57	

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 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]

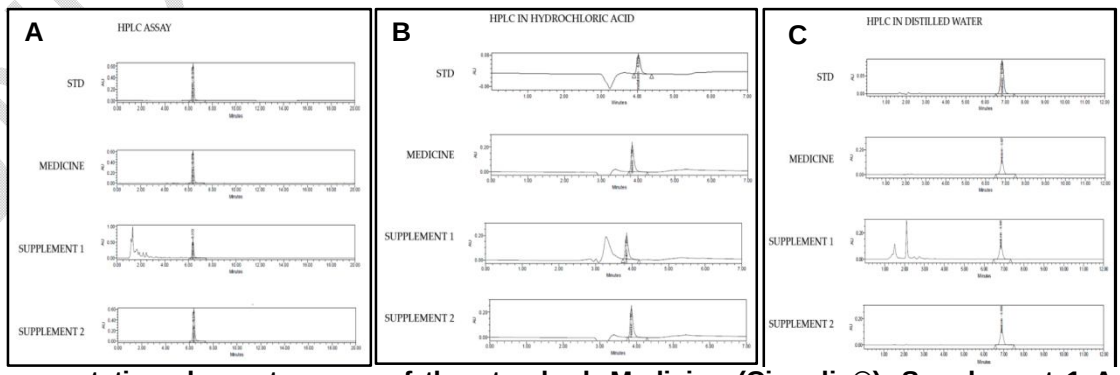


Figure 4. Representative chromatograms of the standard, Medicine (Circadin®), Supplement 1 Aquilea®) and Supplement 2 (Oniria®) in (A) HPLC assay conditions, (B) HPLC in HCl medium and (C) HPLC in distilled water.

Dissolution profiles demonstrated a higher variability between batches in the supplements (Oniria® and Aquilea®) whereas the medicine Circadin® had a very consistent profile in either of the two-dissolution media assayed (Figure 5). As shown in Tables 2 and 3, a higher difference is observed between the batches means of the supplements than in Circadin® batches, which indicates a larger difference between the different batches of the supplements. On the other hand, the RSD (%) values showed that the variability in the tablets of the same batch is lower in medicine batches than in supplements batches.

Table 2. Dissolution profiles of three batches of each product with a distilled water medium (USP).

	Circadin® (Distilled water 500 mL)								
Batch	**80252**			**80361**			**1031665**		
	Dissolved (% of labelled)								
Sample time (min)	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	***R.S.D. (%)***
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	3.9	0.9	22.4	4.1	0.4	9.3	3.2	0.3	9.8
5	10.0	0.6	5.8	9.4	0.4	4.3	7.6	0.3	4.3
10	13.5	0.3	2.4	13.1	0.5	3.8	11.6	0.5	4.2
15	17.9	0.3	1.6	17.7	0.4	2.5	16.1	0.3	2.1
30	24.2	0.4	1.5	23.7	0.7	3.1	22.9	0.7	2.9
45	31.3	0.4	1.3	31.0	0.8	2.5	32.6	5.2	16.0
60	37.1	0.6	1.6	36.0	1.3	3.7	37.3	0.8	2.2
120	47.3	0.8	1.7	46.8	1.2	2.5	49.3	2.0	4.1
180	58.2	0.9	1.6	58.4	1.3	2.2	61.1	2.1	3.4
240	67.5	0.7	1.1	67.5	1.6	2.4	75.6	14.0	18.5
300	74.9	0.7	1.0	74.8	1.8	2.3	82.3	7.3	8.8
360	80.6	0.9	1.2	81.1	1.8	2.2	86.7	1.8	2.1
420	85.7	0.9	1.1	86.0	1.9	2.2	90.4	1.1	1.2
480	89.9	1.1	1.2	90.2	1.8	2.0	94.5	1.5	1.5

	Oniria® (Distilled water 500 mL)								
Batch	**192665**			**193714**			**194540**		
	Dissolved (% of labelled)								
Sample time (min)	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	***R.S.D. (%)***
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	9.8	1.9	19.1	7.8	1.2	15.1	10.0	2.5	25.3
5	21.5	1.8	8.4	22.7	2.8	12.6	21.3	3.8	17.9
10	32.2	2.6	8.1	34.8	3.1	8.9	33.2	2.0	6.0
15	43.7	3.2	7.4	46.1	3.8	8.3	40.5	2.9	7.2
30	52.6	3.6	6.8	56.0	4.7	8.5	47.0	3.0	6.4
45	60.1	4.3	7.2	64.8	5.8	9.0	52.9	3.5	6.7
60	65.1	4.0	6.2	72.7	5.9	8.1	59.7	3.7	6.2
120	72.7	4.5	6.2	83.0	5.9	7.1	70.0	3.3	4.7
180	85.2	4.6	5.4	93.3	5.9	6.4	78.4	3.6	4.6
240	91.1	4.4	4.8	100.4	5.6	5.6	85.2	3.6	4.2
300	95.3	4.3	4.5	103.8	5.3	5.1	89.1	3.7	4.2
360	98.2	4.2	4.3	106.6	5.3	5.0	91.7	3.8	4.1
420	102.0	4.5	4.4	108.6	5.3	4.9	93.4	3.8	4.0
480	104.3	4.4	4.2	110.0	5.4	4.9	95.0	4.0	4.2

	Aquilea® (Distilled water 500 mL)								
Batch	**P014**			**909176**			**910062**		
	Dissolved (% of labelled)								
Sample time (min)	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	***R.S.D. (%)***
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	27.8	9.0	32.3	12.9	4.2	32.4	16.5	3.3	19.9
5	59.3	9.6	16.2	32.1	9.3	29.0	37.8	9.1	24.0
10	70.7	8.1	11.4	42.6	10.7	25.0	49.8	10.9	21.8
15	78.2	6.5	8.3	50.7	11.3	22.2	58.3	11.1	19.0

30	82.6	4.6	5.6	56.2	10.2	18.2	65.2	8.9	13.6
45	87.0	4.3	4.9	60.3	8.8	14.6	70.0	6.8	9.7
60	89.0	3.9	4.4	62.9	8.0	12.7	72.6	5.7	7.8
120	91.6	3.5	3.8	65.1	7.2	11.0	74.7	4.8	6.5
180	93.9	3.2	3.4	66.9	6.1	9.1	76.1	4.6	6.0
240	96.1	3.2	3.3	68.3	5.6	8.2	77.5	4.6	5.9
300	97.5	3.3	3.4	69.4	5.0	7.3	78.2	4.6	5.8
360	98.9	3.4	3.4	71.0	5.7	8.0	79.1	4.6	5.8
420	99.8	3.4	3.4	70.6	4.4	6.3	79.7	4.7	5.9
480	101.1	3.4	3.4	71.7	4.1	5.8	80.5	4.6	5.7

S.D., standard deviation; R.S.D., relative standard deviation

Table 3. Dissolution profiles of three batches of each product with an hydrochloric acid medium.

Circadin® (Hydrochloric acid 0.1M 900 mL)									
Batch	**80252**			**80361**			**1031665**		
	Dissolved (% of labelled)								
Sample time (min)	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	***R.S.D. (%)***
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	8.3	1.3	15.9	4.3	1.0	23.6	3.6	0.5	14.1
5	9.4	0.7	7.1	6.7	1.2	18.4	5.6	0.6	11.0
10	12.8	1.8	14.0	9.7	1.2	12.6	8.5	0.7	8.3
15	16.8	2.1	12.8	13.8	1.1	7.9	12.7	0.8	6.6
30	23.1	3.4	14.9	18.9	0.8	4.5	17.6	0.6	3.6
45	30.5	3.6	11.7	26.4	0.7	2.8	25.3	1.0	3.8
60	37.4	3.3	9.0	33.9	0.4	1.2	33.8	0.8	2.4
120	47.5	6.1	12.7	42.1	1.2	2.7	43.3	1.0	2.3
180	59.0	6.1	10.4	55.0	1.9	3.4	55.7	1.2	2.1
240	69.9	4.5	6.4	65.1	1.9	3.0	67.8	1.0	1.5
300	76.2	3.9	5.1	74.1	1.6	2.2	76.2	1.1	1.4
360	82.0	2.8	3.4	76.0	2.7	3.6	83.2	1.1	1.4
420	86.1	1.6	1.8	85.8	3.9	4.6	87.3	1.1	1.2
480	88.6	1.2	1.3	89.5	5.0	5.6	90.5	1.4	1.5

Oniria® (Distilled water 500 mL)									
Batch	**192665**			**193714**			**194540**		
	Dissolved (% of labelled)								
Sample time (min)	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	***R.S.D. (%)***
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	12.6	1.5	11.8	8.7	2.2	24.8	8.7	2.2	24.8
5	16.1	1.4	9.0	14.2	1.5	10.3	14.2	1.5	10.3
10	23.3	2.0	8.5	20.8	2.1	10.3	20.8	2.1	10.3
15	33.8	3.0	8.8	30.7	2.2	7.1	30.7	2.2	7.1
30	41.9	3.7	8.7	39.6	2.6	6.6	39.6	2.6	6.6
45	52.5	4.2	8.0	50.8	3.4	6.7	50.8	3.4	6.7
60	62.0	4.4	7.1	60.6	4.0	6.6	60.6	4.0	6.6
120	70.7	4.4	6.2	67.3	4.6	6.8	67.3	4.6	6.8
180	79.7	4.7	5.9	77.0	4.8	6.2	77.0	4.8	6.2
240	87.4	5.1	5.8	83.7	4.3	5.2	83.7	4.3	5.2
300	91.7	4.6	5.1	87.6	4.7	5.4	87.6	4.7	5.4
360	94.7	4.7	5.0	89.1	4.8	5.4	89.1	4.8	5.4
420	97.0	5.3	5.5	89.7	5.3	5.9	89.7	5.3	5.9
480	98.7	5.1	5.2	91.8	5.0	5.5	91.8	5.0	5.5

Aquilea® (Distilled water 500 mL)									
Batch	**P014**			**909176**			**910062**		
	Dissolved (% of labelled)								
Sample time (min)	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	**R.S.D. (%)**	**Mean**	**S.D.**	***R.S.D. (%)***
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	39.2	8.0	20.4	9.4	1.5	15.9	16.4	7.2	43.6
5	52.8	7.5	14.1	34.3	5.1	14.9	37.5	6.9	18.3

10	64.2	6.3	9.8	49.5	6.9	13.9	52.1	6.6	12.6
15	74.1	4.8	6.5	59.8	6.1	10.2	62.7	5.5	8.8
30	79.4	3.8	4.8	64.1	5.1	7.9	67.8	4.2	6.1
45	83.7	4.1	4.9	68.1	3.7	5.4	71.6	3.2	4.4
60	86.0	3.8	4.5	70.0	3.2	4.6	74.1	3.2	4.3
120	86.8	3.5	4.0	70.9	3.2	4.6	75.1	3.1	4.2
180	88.2	3.9	4.5	72.2	3.3	4.5	76.9	3.3	4.3
240	88.3	2.6	2.9	73.2	3.0	4.1	77.0	3.3	4.3
300	87.7	4.2	4.8	73.2	3.4	4.7	76.7	3.5	4.5
360	87.0	3.5	4.0	73.5	2.8	3.8	77.0	3.5	4.6
420	87.1	4.1	4.7	73.4	3.0	4.1	76.4	3.4	4.5
480	86.1	3.5	4.1	73.7	3.0	4.0	76.7	3.5	4.5

S.D., standard deviation; R.S.D., relative standard deviation

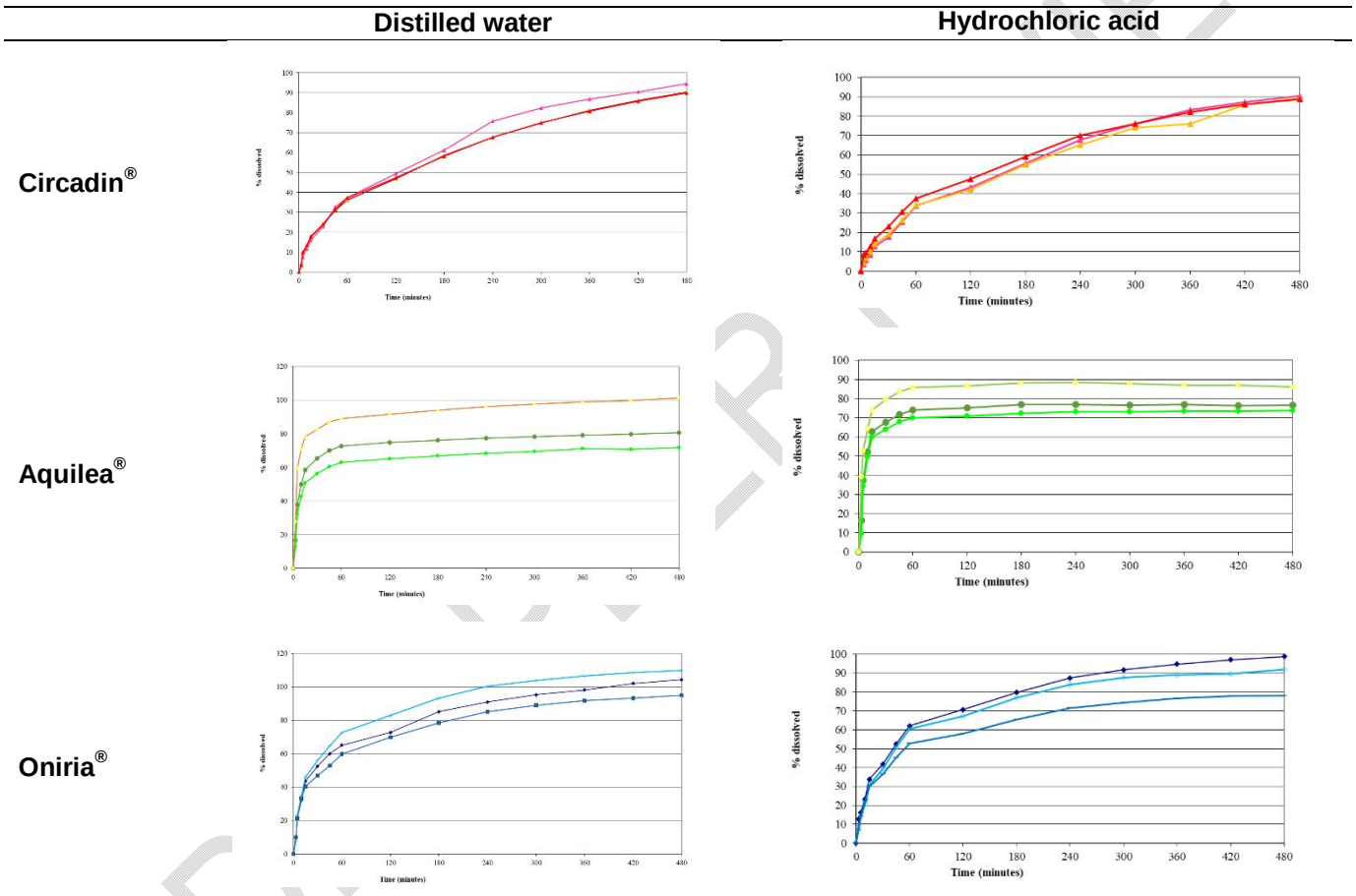


Figure 5. Dissolution profiles of three batches of each product, with distilled water medium (USP) and hydrochloric acid medium.

The medicine Circadin® showed a typical prolonged release profile, showing a release of 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 the content at time 4h levelling off at X2-Y2 % at time 8h. Finally, the Oniria® supplement showed an intermediate profile, considering a melatonin release of >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.

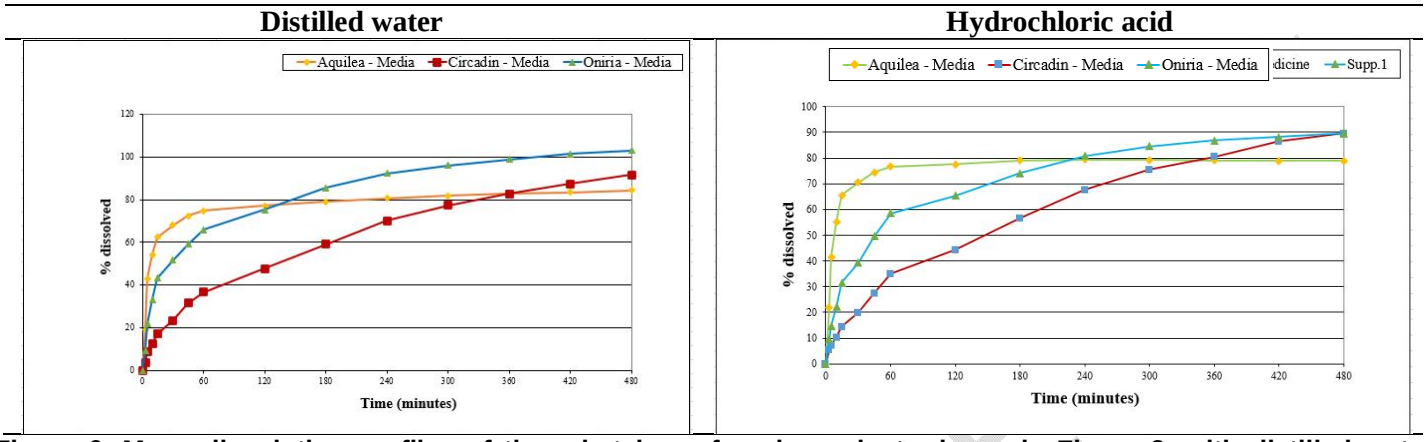


Figure 6. Mean dissolution profiles of three batches of each product, shown in Figure 2, with distilled water medium (USP) and hydrochloric acid 0.1N medium in % of the labelled content.

It is well known that the regulatory pathway, is very strict in the case of medications; they have to stand in the 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 [13]. Hence, some publication alert that food supplements can have a melatonin content significantly lower than the labelled [5] or large variability between lots [14] 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 [15]. 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 number 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 high variability in the release profile, which is important for the efficacy and safety of the product 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 to improve 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 [16]. Among the three melatonin formulations studied in the present work, only the medicine showed a prolonged-release profile in both in vitro dissolution media tested. Specifically, the melatonin medication presented an initial faster release followed by a slow prolonged release of up to eight hours, which is suitable for promoting the induction and maintenance of sleep [12]. 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 by 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 [17-19]. These findings highlight the importance of the exhaustive quality and safety controls required for medications by 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.

REFERENCES

1. Roth, T. Insomnia: Definition, Prevalence, Etiology, and Consequences. J Clin Sleep Med. 2007;3(S5):S7-10.

2. Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol. 2018;175(16):3190-3199.

3. Zisapel N. Melatonin and sleep. Open Neuroendocrinol J. 2010;3:85–95.

4. Lemoine P, Zisapel N. Prolonged-release formulation of melatonin (Circadin) for the treatment of insomnia. Expert Opin Pharmacother. 2012;13(6):895-905.

5. Cerezo AB, Leal A, Alvarez-Fernandez MA, Hornedo-Ortega R., Troncoso AM, Garcia-Parrilla MC. Quality control and determination of melatonin in food supplements. J Food Compos Anal. 2016;45:80-86.

6. Erland LA, Saxena PK. Melatonin natural health products and supplements: presence of serotonin and significant variability of melatonin content. J Clin Sleep Med. 2017;13:275–81.

7. Lelak K, Vohra V, Neuman MI, et al. Pediatric Melatonin Ingestions – United States, 2012-2021. MMWR Morb Mortal Wkly Rep. 2022;71:725-729.

8. Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) [Internet]. Madrid: Ficha técnica o Resumen de las Características del Producto Circadin® (melatonina). [Accessed December 2022]. Available at: https://cima.aemps.es/cima/pdfs/ft/07392003/FT_07392003.pdf

9. Alquilea [online]. Aquilea® Patient leaflet. [Accessed December 2022]. Available at: https://www.aquilea.com/productos/descanso/aquilea-sueno-forte/

10. Italfarmaco [online]. Oniria® Patient leaflet. [Accessed December 2022]. Available at: https://www.italfarmaco.es/media/attachments/2019/11/27/prospecto_oniria.pdf

11. United States Pharmacopeia [online] Melatonin Related Compound A [Accessed December 2022]. Available at: https://static.usp.org/pdf/EN/referenceStandards/certificates/1380116-F0K240.pdf

12. Chua HM, Richer NH, Swedrowska M, Ingham S, Tomlin S, Forbes B. Dissolution of intact, divided and crushed Circadin tablets: Prolonged vs. immediate release. Pharmaceutics. 2016;8:2.

13. Hahm H, Kujawa J, Augsburger L. Comparison of melatonin products against USP's nutritional supplements and other criteria. J Am Pharma Assoc. 1999; 39(1):27-31.

14. Erland LA, Saxena PK. Melatonin natural health products and supplements: presence of serotonin and significant variability of melatonin content. J Clin Sleep Med. 2017;13:275–81.

15. Glass J, Lanctôt KL, Herrmann N, Sproule BA, Busto UE. Sedative hypnotics in older people with insomnia: meta-analysis of risks and benefits. BMJ. 2005;331(7526):1169.

16. Lemoine P, Zisapel N. Prolonged-release formulation of melatonin (Circadin®) for the treatment of insomnia. Expert Opin Pharmacother. 2012;13(6):895-905.

17. Ferracioli-Oda E, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One. 2013;8(5):e63773.

18. Salanitro M, Wrigley T, Ghabra H, et al. Efficacy on sleep parameters and tolerability of melatonin in individuals with sleep or mental disorders: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2022;139:104723.

19. Grima NA, Rajaratnam SMW, Mansfield D, et al. Efficacy of melatonin for sleep disturbance following traumatic brain injury: a randomised controlled trial. BMC Med. 2018;16(1):8.