Original Research Article

Formulation, stability testing, and analytical characterization of melatonin-based preparation for clinical trial

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A B S T R A C T

A new institutional clinical trial assessed the improvement of sleep disorders in 40 children with autism treated by immediate-release melatonin formulation in different regimens (0.5 mg, 2 mg, and 6 mg daily) for one month. The objectives of present study were to (i) prepare low-dose melatonin hard capsules for pediatric use controlled by two complementary methods and (ii) carry out a stability study in order to determine a use-by-date. Validation of preparation process was claimed as ascertained by mass uniformity of hard capsules. Multicomponent analysis by attenuated total reflectance Fourier transformed infrared (ATR-FTIR) of melatonin/microcrystalline cellulose mixture allowed to identify and quantify relative content of active pharmaceutical ingredients and excipients. Absolute melatonin content analysis by high performance liquid chromatography in 0.5 mg and 6 mg melatonin capsules was 93.6% ± 4.1% and 98.7% ± 6.9% of theoretical value, respectively. Forced degradation study showed a good separation of melatonin and its degradation products. The capability of the method was 15, confirming a risk of false negative < 0.01%. Stability test and dissolution test were compliant over 18 months of storage with European Pharmacopoeia. Preparation of melatonin hard capsules was completed manually and melatonin in hard capsules was stable for 18 months, in spite of low doses of active ingredient. ATR-FTIR offers a real alternative to HPLC for quality control of high-dose melatonin hard capsules before the release of clinical batches.

1. Introduction

Autism is a life-long neurodevelopmental disorder, interfering with a person's ability to communicate and relate to others. Heterogeneous expression of autism involves severe symptoms and related disorders with a prevalence estimated at 62 cases per 10,000 people [1]. Sleep disturbances are highly prevalent in individuals diagnosed with autism spectrum disorder (44%–83% of school-aged children [2]) and are major contributors to poor daytime functioning in these individuals. Pre-sleep arousal in autism related to daytime stress may significantly delay sleep onset, and lead to an insomnia [3]. Deficit of melatonin activity would contribute to trouble sleeping [4]. Melatonin is a hormone produced by the pineal gland in response to a decrease of the light intensity [5] and its structure is close to that of serotonin (Fig. 1). Physiologically, melatonin secretion increases soon after nightfall, and reaches a maximum between 2:00–4:00 after midnight and then decreases. The activity of melatonin receptors (MT1, MT2 and

MT3) is known to contribute to its properties of sleep facilitation, since MT1 and MT2 receptors participate in the control of circadian rhythms and regulation of nycthemeral cycle. A commercial medication of melatonin (Circadin® LP 2 mg, melatonin extended release) is available at a single dose, enabling slow absorption and sustained pharmacological activity. Several randomized controlled studies showed the effectiveness of melatonin on reducing sleep disturbances in children with atypical development [5–9]. An earlier study involving children with pervasive developmental disorders showed that immediate-release melatonin was more effective among children with late sleep, but the sleep maintenance was better with extended-release form [10]. None of these studies investigated the dose-effect relationship of melatonin on sleep patterns. In the present study, a randomized double-blind institutional clinical trial was designed to study the improvement of sleep disorders in 40 children with autism treated by immediate-release melatonin formulation in different regimens (0.5 mg, 2 mg and 6 mg daily) for one month. As part of the technical
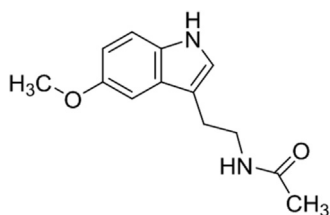
Fig. 1. Chemical structure of melatonin.

feasibility analysis of a new institutional clinical trial on melatonin, hospital pharmacy was involved in the production of immediate-release melatonin capsules and the achievement of quality control according to good preparation practices [11]. The general purpose of the present study was to prepare a melatonin-based formulation that was not available on the market. More specifically, a stability study was carried out according to the recommendations of the International Conference on Harmonization (ICH) [12,13] to set an expiry date, including the validation of a novel stability-indicating high performance liquid chromatographic (HPLC) method for the assay of melatonin in bulk and pharmaceutical formulation (presence of excipients) [14], multi-component infrared analysis and dissolution testing.

2. Experimental

2.1. Chemicals and drugs

Melatonin, also known as *N*-acetyl-5-methoxytryptamine or *N*-[2-(5-methoxyindol-3-yl)ethyl], is synthesized from tryptophan. Its chemical structure is close to the serotonin (Fig. 1). Physicochemical properties of melatonin are reported in Table 1. Melatonin is sensitive to light and oxidation. Melatonin is a small non-polar and amphiphilic molecule which has the ability to penetrate the blood-brain barrier. Melatonin powder (purity > 99%; Inresa®, Bartenheim, France) and microcrystalline cellulose powder (Cooper®, Melun, France) were used as standards during analytical validation and for clinical batches. Circadin® LP 2 mg is a commercial medication provided by Neurim® Pharmaceuticals (Baarerstrasse, Switzerland). Acetonitrile and methanol (Merck Millipore®, Darmstadt, Germany) were all of HPLC grade. Purified water (Lavoisier®, Paris, France) was used for all purposes. Before injection, all solutions were filtered through polytetrafluoroethylene filter (Millex®, 0.22 μm, Merck Millipore®, Darmstadt, Germany). Monopotassium phosphate (Sigma-Aldrich®, Missouri, United States) and sodium hydroxide (Acros Organics®, Geel, Belgium) powders were used for the preparation of dissolution medium.

2.2. Instrumentation

An HPLC Agilent® (Agilent® 1290 Infinity Quaternary LC System, Les Ulis, France) equipped with a binary pump with integrated vacuum degasser, a thermostated column compartment, an autosampler and a diode array detector was used for analysis of melatonin content. The results were collected and evaluated statistically using HP

ChemStation® software. Attenuated total reflectance Fourier transformed infrared spectroscopy (ATR-FTIR) spectra of powdered samples (pure raw materials and microcrystalline cellulose and melatonin mixtures) were recorded in Nicolet® iS 50 FT-IR Spectrometer (Thermo Scientific®, Thermo Nicolet Corp.®, Waltham, MA, USA) applying ATR method with diamond crystal and having a standard deuterated triglycine sulfate (DTGS) detector (optical path: 10 cm). For each analysis, a total number of scans was 32 with spectral resolution of 4 cm^{-1}. Omnic Spectra® software was used to process simplified and multi-component spectra. Dissolution testing was performed with a USP dissolution tester Pharmatest DT 70® with paddles (Pharmatest Apparatebau AG®, Hainburg, Germany) equipped with seven stirred positions. For sampling, guiding tubes were placed into the seven holes in the top cover of the instrument. The system ensured that all samples were taken from the same position in the testing vessel.

2.3. Preparation of melatonin hard capsules

Three pilot batches of melatonin hard capsules (N° 4, ivory, gelatin) were prepared by mixing in a mortar and pestle: (i) 0.5 mg of melatonin with 77 mg of microcrystalline cellulose, (ii) 2 mg of melatonin with 75.5 mg of microcrystalline cellulose and (iii) 6 mg of melatonin with 71.5 mg of microcrystalline cellulose. The conditioning of melatonin/microcrystalline cellulose mixture in hard capsules was carried out manually by pharmaceutical staff.

2.4. Stability study of melatonin hard capsules

Stability of 0.5 mg and 6 mg melatonin hard capsules (n=300) prepared as detailed previously was investigated for 18 months according to ICH Q1A (R2) [12]. Melatonin hard capsules were kept in a climatic chamber (Meditest 1300 H®, Froilabo®, Meyzieu, France) maintained at 25 ± 2 °C and 60% ± 5% relative humidity (RH) as recommended in ICH Q1A (R2) [12] for long term storage. The acceptance criteria of stability study, including melatonin assay by HPLC, physicochemical stability, multicomponent infrared analysis and dissolution testing, was realized immediately after preparation (day 0), and then after 3, 6, 9, 12, 15 and 18 months of storage in climatic chamber.

2.4.1. Melatonin chromatographic assay

European Pharmacopoeia, the 8th edition (Eur. Ph. 8) has no monograph for melatonin. [15], while a monograph was reported in the German Drug Codex [16]. In the present study, chromatographic conditions for melatonin assayed were adapted from German Drug Codex (Deutscher Arzneimittel Codex, Supplement to the Pharmacopoeia, DAC 2009, M-080) including reverse phase C_{18} column (150 mm×4.6 mm, 3 μm; Luna Phenomenex®, Le Pecq, France) thermostated at 40 °C, and mobile phase composed of water/acetonitrile mixture (60:40, v/v). Sample volume was 10 μL, the mobile phase flow rate was set at 1 mL/min, and detection was made at 280 nm. Standard melatonin solutions ranging from 6 to14 μg/mL were prepared by dissolving melatonin in water/acetonitrile mixture (60:40, v/v). In order to appreciate the influence of microcrystalline cellulose upon melatonin detection, standard melatonin solutions (1 mg/mL) were supplemented by suspending microcrystalline cellulose (1.5 mg/mL), which was dispersed ultrasonically for 15 min, and then centrifuged. Finally, supernatants were filtered (Millex® filter 0.22 μm) before HPLC assay. The analytical validation was performed according to the recommendations of the ICH Q2B [13] including the assessment of system suitability, specificity, linearity, accuracy, precision (repeatability, intermediate precision), limits of detection and quantification (LOD and LOQ) (see more details in Supplementary material), robustness and capability. The system suitability was determined from replicate injections of standard melatonin solution and all melatonin hard capsule samples, in normal and forced

Table 1

Parameters	Melatonin
Molecular mass	232.28 g/mol
Log P (octanol-water)[a]	1.65
Solubility (25 °C)	
In water	0.1 mg/mL
In ethanol	50 mg/mL
pKa	4.4
Color	Crystalline ivory beige

[a] ChemID*plus* (website: http://chem.sis.nlm.nih.gov/chemidplus/)

degradation conditions in order to demonstrate the system's separation capability. Retention time, the number of theoretical plates, tailing and capacity factors were selected as chromatography parameters for testing system suitability [17]. Standard melatonin solutions with or without excipient were subjected to forced degradation by adding 5 M HCl solution at 70 °C for 30 min, 2 M NaOH solution at 70 °C for 30 min, 3% H_2O_2 solution at 80 °C for 10 min or by applying UV (λ=365 nm) for 5 h. Robustness of the method was tested by varying chromatographic experimental conditions (i.e., flow rate (± 10%), solvent mixture (± 3.3%) and column compartment temperature (± 5%)) during analysis of melatonin standard solution (10 µg/mL) (n=5). The capability allowed to assess the risk of false negatives that can be generated by a validated analytical method and was calculated as follows [18]:

Cp =[difference between the highest and lowest limits of confidence interval]/6σ.

A simple calculation of the capability of the method allowed to estimate the risk of false negative results and performance of the method [18].

2.4.2. Physicochemical stability

The physical stability of melatonin hard capsules was evaluated by visual inspection allowing the detection of hardening or softening of shell, coloration, aggregation, and compaction of content. The uniformity of mass (Eur. Ph. 8, Monograph 2.9.5) [15] was evaluated by weighing individually 20 capsules (Cubis MSU, Sartorius, France). The uniformity of melatonin content in hard capsules (Eur. Ph. 8, Monograph 2.9.6) [15] was assayed by dissolving melatonin/microcrystalline cellulose mixture contained in hard capsule (n=20) in 50 mL of water/acetonitrile mixture (60:40, v/v), and then filtered prior to analysis by HPLC. The acceptance criteria included unchanged macroscopic appearance of capsule and the variation of melatonin hard capsule mass and melatonin content restricted to ± 10% of theoretical value.

2.4.3. Multicomponent infrared analysis

The linearity of assay by ATR-FTIR (3800–400 cm^{-1}) was tested in triplicate by using standard melatonin/microcrystalline cellulose mixture ranging from 2 to 36 mg prepared on account of a range day per operator. A separated control of raw material (melatonin/microcrystalline cellulose) relative content was also performed. Treatment of a spectrum obtained by the analysis of melatonin/microcrystalline cellulose mixture ATR-FTIR spectrum was proceeded with the Omnic spectra® software in multicomponent mode in which the first compound (major compound) was identified by simply comparing intensities/amplitudes across similar wavelength ranges between the spectrum obtained and a reference spectrum contained in the database by converting spectra into vectorial forms and comparing the vectors. Then, the spectrum obtained was compared with a new spectrum generated by the software combining the spectrum of the compound identified previously and the spectrum of another compound. The new generated spectrum takes into account the weight of each spectrum, by a weighted regression analysis, thus providing the best match and a quantitative analysis [19]. An infrared spectral analysis of melatonin, microcrystalline cellulose and melatonin/microcrystalline cellulose mixture was carried out. Multicomponent mode analysis of melatonin/microcrystalline cellulose infrared spectrum allowed to identify both melatonin and microcrystalline cellulose and to estimate melatonin/microcrystalline cellulose respective content.

2.4.4. Dissolution testing

Dissolution testing of melatonin hard capsules (n=6) was performed by using a USP dissolution tester, Pharmatest DT 70® with paddles (Pharmatest Apparatebau AG®, Hainburg, Germany). Dissolution medium was composed of 50 mM phosphate buffer (pH 6.8) prepared by mixing 0.2 M KH$_2$PO$_4$ (250 mL) and 0.2 M NaOH (112 mL) solution in purified water (638 mL) (Eur. Ph. 8, Monograph 2.9.3) [15]. The dissolution of 0.5 mg and 6 mg melatonin hard capsules was assayed in 60 mL and 500 mL of dissolution medium, respectively, taking account the limit of melatonin quantification of HPLC method. The temperature of dissolution medium was maintained at 37 ± 2 °C and paddles rotation was settled at 50 rotations per minute. Samples of dissolution medium (1 mL) were collected at different intervals of time (0, 2.5, 5, 7.5, 10, 20 and 30 min), and then replaced by equal volume of fresh dissolution medium. Melatonin content in the sample was analyzed by HPLC. The acceptance criteria specified that 85% of melatonin contained in hard capsule must be recovered in dissolution medium after 30 min. If the release of melatonin content of six capsules was lower than 85% within 30 min, the melatonin release of six supplementary capsules was tested.

2.5. Assay of melatonin content in hard capsules and in commercial medication

Melatonin content in ten melatonin hard capsules (0.5 and 6 mg) at random on a batch of 300 capsules was analyzed. Likewise, ten tablets of Circadin® LP 2 mg were assayed after being crushed and dissolved in water/acetonitrile mixture (60:40, v/v) [20]. The solution was then sonicated for 15 min and centrifuged, and finally the supernatants were diluted (1/100) and filtered through 0.2 µm PTFE filter (Merck Millipore®, Darmstadt, Germany).

2.6. Statistical analysis

All statistical tests were performed by the Excel® software (Microsoft Office, USA, 2007) with a risk α of 5%. The linearity was evaluated by a linear regression model estimated by the method of least squares. The peak area intercept of regression line was assessed by Student's t test. The verification of the homogeneity of variance was performed by Cochran's test. The test for determining the existence of a significant slope was made by a Fischer-Snedecor's test. The accuracy was tested by using Cochran's and ANOVA tests. The confidence interval of the average recovery was determined by Student's t test. Finally, the precision was validated by Cochran's test. The coefficients of variation were calculated for intermediate precision and repeatability.

3. Results and discussion

Given the lack of adapted drug products for pediatric use, the preparation of melatonin oral capsules, suitable for this clinical trial, was mandatory to ensure proper medical care for children with autism. The drugs administered orally were made available as capsules at three different doses of 0.5 mg, 2 mg and 6 mg. This oral dosage form permitted a quick dispensation for hospitalized and ambulatory patients. Physical and microbiological stability of a liquid oral form was short, requiring conservatives difficult to get for the pediatric population. In addition, the capsules were made at low dosages, allowing the administration to children without fractionation by nurses or parents. The manufacture of these preparations involved many steps, each of which can be the cause of a failure. Indeed, the method of nonindustrial preparation capsules may contain errors due to the human factor, especially upon mixing, distribution, and conditioning done manually. These errors due to manual preparation might be more important for the lowest dose of melatonin. Thus, to ensure proper melatonin dosage for each manufacturing, quality control of raw material due to variability in the quality of the powder, mixture and the active principle content in the capsules was made according to the recommendations of Eur. Ph. 8 [15].
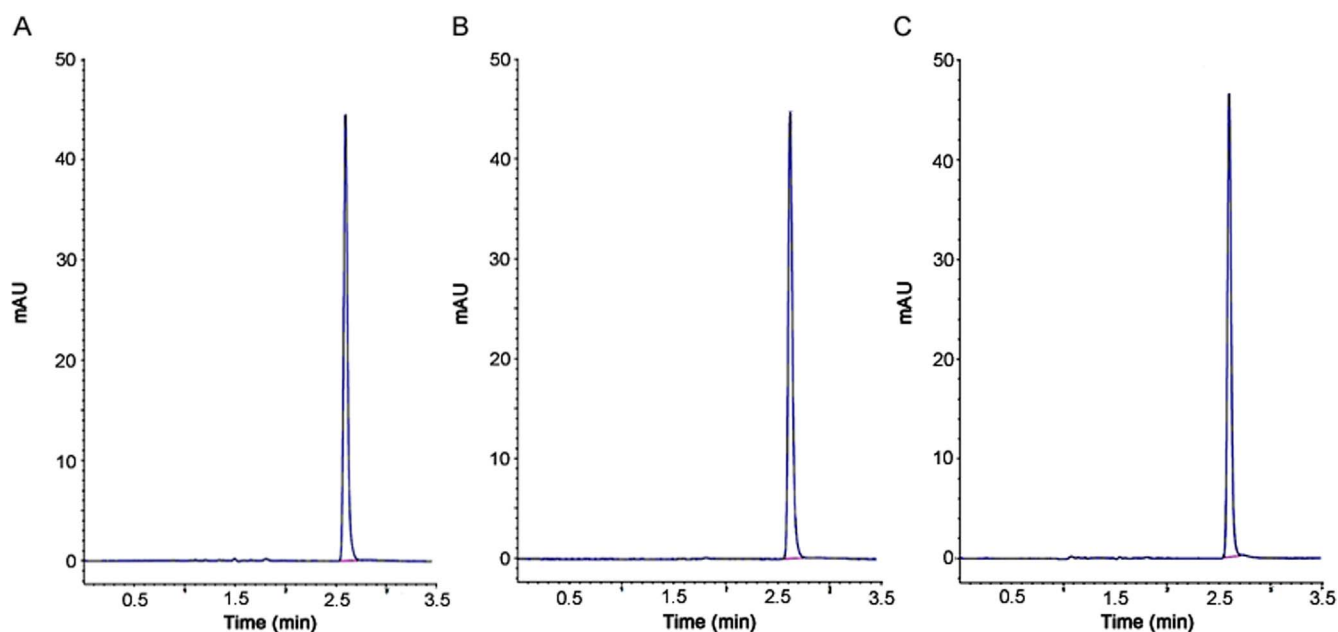
Fig. 2. Chromatograms of (A) melatonin solution, (B) melatonin/ microcrystalline cellulose suspension and (C) Circadin® LP, 2 mg obtained by HPLC.

3.1. Stability study of melatonin hard capsules

3.1.1. Melatonin chromatographic assay

Validation of melatonin chromatography assay was conducted in accordance with ICH guidelines (Q2B) [13]. Therefore, the system suitability tests checking the performance of analysis systems before and during routine analysis were performed automatically for each analysis (Table S1). According to the German Drug Codex of melatonin, the areas of individual and all secondary peaks should not exceed 0.1% and 0.5% of the peak area of melatonin, respectively. In addition, resolution of melatonin and impurities peaks and signal/noise ratio should be higher than 10 and 4, respectively [21]. Acceptance criteria specified in the German Drug Codex have been met [16]. The specificity of the HPLC method is illustrated in Figs. 2A and B where complete separation of melatonin was noticed in presence of excipient. Chromatograms of melatonin solution and melatonin/microcrystalline cellulose mixture showed no interference between active drug product and excipient. Analysis of peak purity by using photodiode array showed that purity angle was always less than purity threshold for the analyte. In basic and acidic media, peak area of melatonin decreased concomitantly to the increase of degraded product separated from melatonin peak. Similarly, in the presence of H_2O_2 or under UV, degradation products were observed. Fig. 3 shows the chromatograms of melatonin subjected to forced degradation conditions. For the achievement of calibration curves, five standard solutions of melatonin solution and melatonin/microcrystalline cellulose suspension were prepared in the range of 6−14 µg/mL. The results, summarized in Table 2, showed a good correlation between analyte peak area and concentration with $r > 0.993$ ($n=5$). According to the *Student's t* test [22], the peak area intercepts of regression line of melatonin solution and melatonin/microcrystalline cellulose suspension were not significantly different ($p > 0.05$) (Table 2). Similarly, the slopes of the peak area-melatonin concentration curve of melatonin solution and melatonin/microcrystalline cellulose suspension were not significantly different ($p > 0.05$), confirming the absence of matrix effect related to the excipient and the absence of systematic error related to the method (Table 2). Standard melatonin solution (10 µg/mL) could be used as a reference for the stability study or regulatory quality control of clinical batches. The average recovery rate was calculated for each concentration. The average recovery of melatonin solution and melatonin/

microcrystalline cellulose suspension was 99.3% and 101.2%, respectively, with a confidence interval ranging from 98.8% to 101.7% and from 99.6% to 102.6%, respectively, confirming the accuracy of the method and the absence of excipient analytical interaction. Repeatability and intermediate precision of chromatography assay are shown in Table S2. Variations in the repeatability and intermediate precision were objectified by calculating RSD of six experimental measurements. The precision was performed by using a solution of melatonin of 10 µg/mL. The variability of repeatability and intermediate precision was lower than 4% (RSD of repeatability: 2.6%; RSD of intermediate precision: 3.2%), confirming the precision of chromatography assay. LOD and LOQ of melatonin solution were 1.14 µg/mL and 3.50 µg/mL, respectively. LOD and LOQ of melatonin/microcrystalline cellulose suspension were 1.01 µg/mL and 3.08 µg/mL, respectively, with similar selectivity of 1.30, confirming the sensitivity of chromatography assay. The results of the robustness study are summarized in Table S3. The robustness study demonstrated that small variations, deliberately introduced into the parameters of the method, did not affect significantly the proposed method for dosing the melatonin. This provided an indication of its reliability during routine analysis. This study also set limits compliance testing. Thus, any changes caused a significant change in the retention time or the area under the curve (for theoretical plates, tailing factor). No change over 15% was observed under three different conditions with two variation levels. It should be emphasized that the changes at the mobile phase flow rate had modified more significantly the retention time than in other conditions. The capability (15) of the method was found much higher than 1.33, confirming its high performance. The risk of false negative results was estimated to be < 0.01%. The present chromatographic method was fast (melatonin retention time < 3 min), and needed a small sample injection volume, without buffering or acidification of mobile phase as compared to previous methods (Tables S4 and S5) [23-27]. Moreover, the absence of a buffer in the mobile phase, as used in some methods, reduced the risk of crystallization (no salt deposition on the seals of the pump or the level of the injector), preserving the longevity of the column, and without requiring any guard column. Furthermore, it was noted that the wavelengths between the various methods were not the same and ranged from 223 to 304 nm. The optimum wavelength was selected after analysis of the spectrum of melatonin by multi-wavelength using a diode array
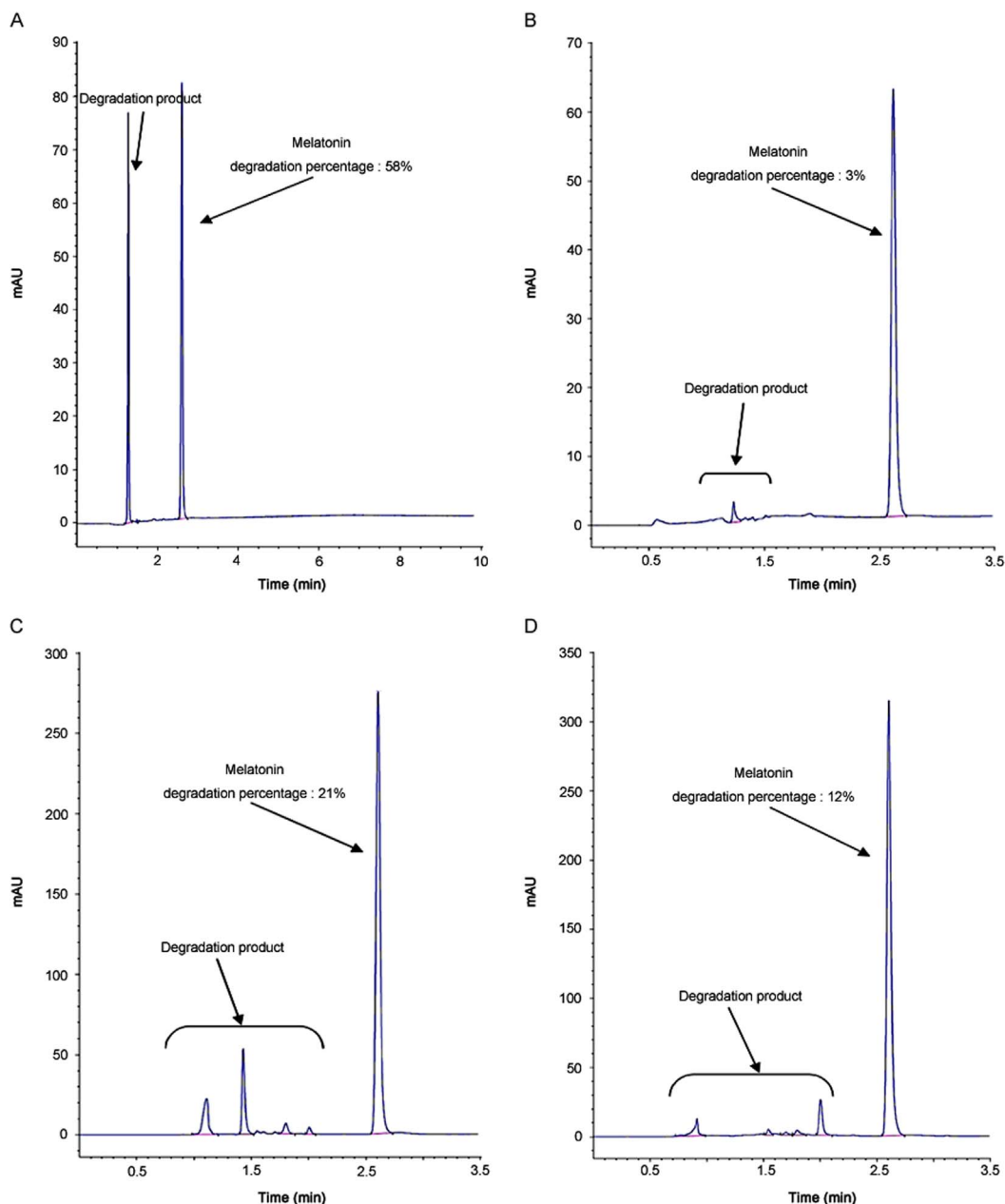
Fig. 3. Chromatograms of melatonin after forced degradation by (A) 5 M HCl solution at 70 °C for 30 min, (B) 2 M NaOH solution for 30 min at 70 °C, (C) 3% H_2O_2 solution at 80 °C for 10 min and (D) UV (λ=365 nm) for 5 h.

Table 2

Linear regression analysis of melatonin assay by HPLC and comparison of intercept and slope of melatonin solution (10 µg/mL) and melatonin (10 µg/mL)/microcrystalline cellulose (15 µg/mL) suspension.

Parameters	Melatonin	Melatonin/microcrystalline cellulose
Regression analysis		
Linearity range (µg/mL)	6–14	6–14
Slope (mAU*min*mL/µg)	12.68	12.21
Intercept (mAU*min)	−2.58	0.68
Correlation coefficient (r)	0.993	0.994
Comparison of intercepts [18]		
s_a (mAU*min*mL/µg)[a]	4.40	3.76
Comparison of slopes [18]		
s_b (mAU*min)[b]	0.42	0.36

[a] P=0.2
[b] P=0.08

detector (Fig. 4). The wavelength chosen was 280 nm, which corresponded to that mentioned in the German Drug Codex monography of melatonin, specifically at 278 nm. The comparison between different forced degradation studies could not be performed because the conditions were different from one study to another. It is worth noting that the combination of thermal hydrolysis with 3% H_2O_2 degraded most of the melatonin. Thus, no degradation was observed at 25 °C whereas at 90 °C 40% of the melatonin was degraded after 2 h. The chromatograms of forced degradation studies were not reported in other publications, while chromatographic purity was missing in earlier studies [23–27]. The validation according to the ICH guidelines ensured the reliability, accuracy and precision of chromatography assay during a routine analysis. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. Moreover, the proposed reverse phase-HPLC method was found to be simple, rapid, sensitive, precise, cost-effective, accurate, and stability-indicating [28,29].
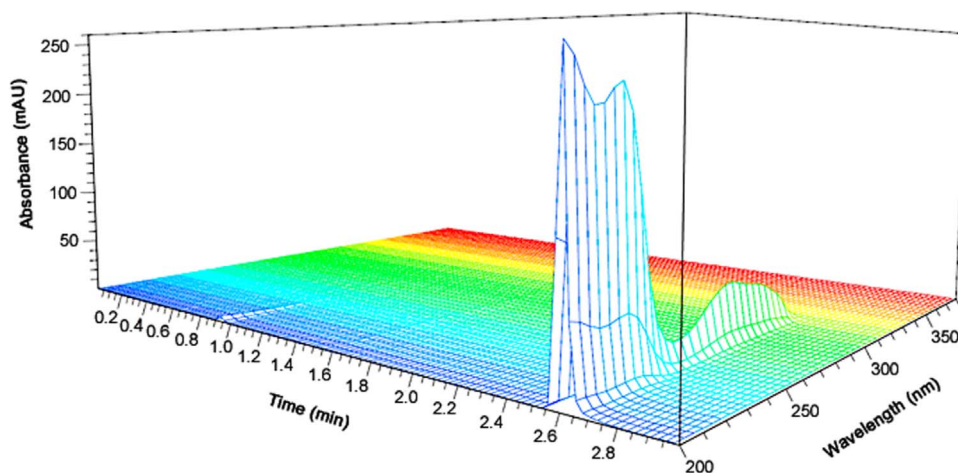
Fig. 4. Multi-wavelength detection of melatonin by HPLC.

3.1.2. Physicochemical stability

The physicochemical controls of melatonin capsules are reported in Table S6. There were no detectable variations of color, odor, shape, and consistency of capsules. Weight of melatonin capsules and melatonin content showed acceptable variation (< 10%, Eur. Ph. 8, Monograph 2.9.6 [15]). After 18 months, the melatonin content in 0.5 mg and 6 mg melatonin capsules was 93.6% ± 4.1% and 98.7% ± 6.9% of theoretical value, respectively.

3.1.3. Multicomponent infrared analysis

Multicomponent ATR-FTIR spectra analysis allowed both separated identification of melatonin and microcrystalline cellulose and estimation of melatonin/microcrystalline cellulose relative content in 2 mg (5.9:94.1, m/m) and 6 mg (9.4:90.6, m/m) melatonin capsules (Fig. 5A). Interestingly, a linear relationship was found between the relative content of melatonin/microcrystalline cellulose determined by ATR-FTIR and the mass ratio of melatonin (0%–100%) and microcrystalline cellulose (0%–100%) (Fig. 5B). After 18 months of storage, ATR-FTIR spectra of melatonin/microcrystalline cellulose mixture showed no significant difference compared to that recorded immediately after preparation, confirming the absence of active pharmaceutical ingredient and/or excipient degradation, or exogenous contamination such as water sorption. The multicomponent infrared analysis is a quick and cost-effective technique to check the proportions of components in the capsules. The comparison of the two methods (HPLC and ATR-FTIR) is reported in Table S7. This rapid analytical technique, requiring no reagent, should be exploited in routine control of production batches. Indeed, for the capsules consisting of two components, the combination of mass uniformity with the determination of the proportion of each component may deduce the content of each component in the capsule in a very short time. The percentage error obtained by deduction in ATR-FTIR between the melatonin content to 18 months and theoretical melatonin content was correlated with the percentage error obtained by HPLC (−1.3%). The detection of impurities in pulverulent mixture as recommended in the Eur. Ph. 8. [15] can also be made by ATR-FTIR, as reported previously by assaying impurities of piracetam [30]. Only the determination of degradation products remains unfeasible by ATR-FTIR, since these degradation products are not known. Similarly, the forced degradation study, performed with solutions (acid, alkaline, oxidant), cannot be analyzed by ATR-FTIR due to the interference of water background. However, the appearance of degradation products in the stability study would induce a decrease in proportion to melatonin. However, ATR-FTIR was not sensitive enough to estimate low melatonin dosage. This limitation could be overcome by FTIR analysis of melatonin and microcrystalline cellulose in KBr pellets.
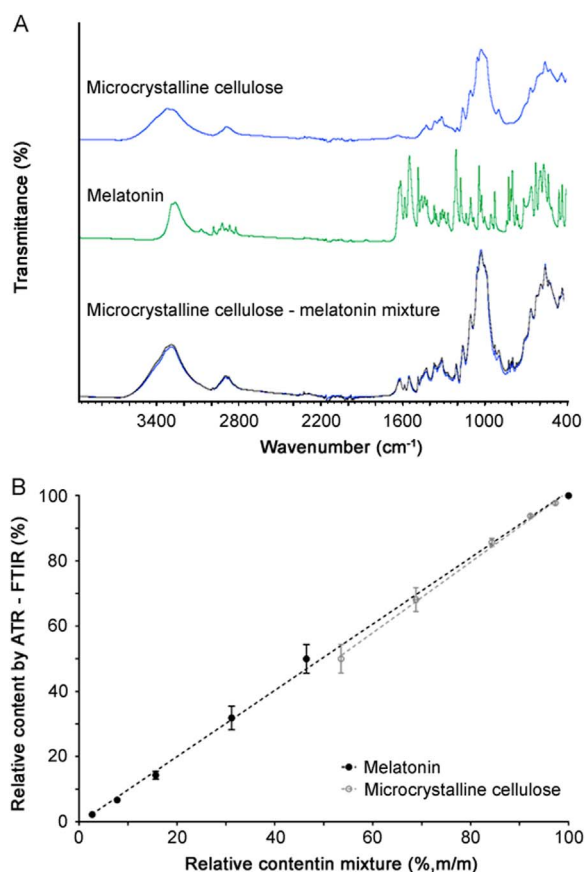

Fig. 5. (A) Simultaneous determination of melatonin and microcrystalline cellulose relative content in a 6 mg melatonin hard capsule by ATR-FTIR. Upon treatment of melatonin/microcrystalline cellulose spectrum, melatonin and microcrystalline cellulose were identified separately and matched closely original components (melatonin: 94%; microcrystalline cellulose: 73%) and the relative content of melatonin and microcrystalline cellulose in mixture was resolved (melatonin:microcrystalline cellulose: 9:91, m/m). (B) A linear relationship was found between the relative content of melatonin/microcrystalline cellulose determined by ATR-FTIR and the mass ratio of melatonin (0%–100%; R = 0.9989) and microcrystalline cellulose (0%–100%; R = 0.9984). Each data point is the mean ± standard deviation of three determinations.

3.1.4. Dissolution testing

The recovery of melatonin after 18 months of storage from 30 min dissolution test was found higher than 85% in accordance with Eur. Ph. 8, Monograph 2.9.3 [15]. The dissolution test ensured that the release of

melatonin contained in hard capsule after ingestion was satisfactory in accordance with Eur. Ph. 8 [15].

Neither modification of hard capsule nor degradation of melatonin in hard capsule was observed over 18 months of storage at 25 °C /60% RH. Thus, the use-by-date of these melatonin hard capsules, kept away from the light, was set at 18 months at 25 °C /60% RH. However, the practical use of melatonin capsules for autistic children involved an opening of the capsule. Therefore, the content is withdrawn and mixed with food or drink products which might potentially modify melatonin stability. All these steps of formulation development, preparations and control taking into account various factors including features of pediatric populations and current regulations show the interest of compounding unit in a clinical trial.

3.2. Assay of melatonin content in hard capsules and in commercial medication

The adequacy of chromatography assay was investigated by determining the melatonin content in hard capsules and commercial medication (Circadin® LP 2 mg). The chromatogram of commercial medication is presented in Fig. 2C and the amount of melatonin was determined from the calibration curve. The results, in agreement with the label claims, are presented in Table S8. There was no interference with microcrystalline cellulose or other excipients of commercial medication Circadin® LP 2 mg (ammonium methacrylate copolymer type B, dicalcium phosphate dihydrate, lactose monohydrate, colloidal anhydrous silica, talc, magnesium stearate). The spectral information was also used for identification of the compound compared with the UV spectrum of the pure compound. The peak purity was estimated at 99.9%, justifying the absence of co-elution.

4. Conclusion

Preparation of melatonin hard capsules was completed manually in spite of low doses of active ingredient. The regulatory quality control of hard capsules was established before the release of clinical batches as part of the clinical trial by physicochemical controls, stability study and dissolution test. Two methods were developed and validated for the pharmaceutical control of melatonin hard capsules. The benefits of multicomponent infrared analysis in discharging quality control of hard capsules have been pointed out. A restriction of use of this melatonin-based preparation was set at 18 months at room temperature and away from the light.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpha.2017.04.001.

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