The Influence of Storage Conditions on Melatonin Stability

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Abstract **- Stability of melatonin in different storage conditions has been determined. The choice of the best storage condition in this work is based on an analysis if melatonin stability may suffer from stress in the presence of light, air, lower and high temperature. It has been found as melatonin has an acceptable stability in all storage conditions tested: Darkness at -32ºC, darkness at 4ºC, darkness at 25ºC, darkness at 50ºC, light at -32ºC, light at 4ºC, light at 25ºC and light at 50ºC until day 13, except the samples kept at room temperature in light and in darkness without air protection; which degrades strongly from the beginning (day 2) to reach more than 41% on the sixth day and more than 66% on the fifteenth day.**

Keywords- melatonin, stability, degradation.

I. INTRODUCTION

Melatonin (N-acetyl-3-(2-aminoethyl)-5-methoxyindole), a neurohormone produced by the pineal gland, can also be found in foods of plant origin. Melatonin has been proved to act as a very biologically active compound, so it has increased substantially the interest of their study in different types of plants and fruits. This molecule is a biogenic indoleamine, which performs an important role for example in the regulation of circadian rhythm **[1]**, also in the reduction of sleep disorders, including insomnia due to jet-lag and shift work [2];[3]. It has also been reported to have potent anti-oxidative properties and anti-inflammatory effects [4]; [5]. Additionally, some protective effects in ocular diseases has also been described [6].There are also studies showing some anticarcinogenic properties for melatonin [7].

Some literature reviews suggests that the accumulation of melatonin in reproductive tissues, seeds and fruits, may be indicative of a mechanism for protection of the tissues from oxidative damage arising from drought, cold, heat, UV light, or environmental toxins (7).

The analysis of melatonin is a difficult task as it occurs in low ppb levels in foods. Various analytical methods have been used to analyse melatonin in foods. These include liquid chromatography with different detectors: electrochemical [8], fluorimetric [11], and MS /MS. [13]; [14], and others immunological techniques such as

radioimmunoassay (RIA), enzyme-Immunoassay (EIA) [15], and immunoprecipitation [16]. Another possible alternative is capillary electrophoresis [17] in different variants, which has the advantage of low values of limits of quantification warn that the matrix effect of plant samples due to the presence of reducing agents may interfere in the analysis.

The chromatographic techniques are more economical and time efficient when derivatisation of the sample is not required prior to analysis. Most of the HPLC methods reviewed have used reverse phase columns RP_{18} or RP_8 for melatonin separation and fluorescence detectors were found to be sensitive and versatile to quantify melatonin in food samples and also gave low limits of detection and quantification [18].

For the application of these methods, and determination of melatonin in foods; is usually necessary step prior to sample preparation (sample pre-treatment); in fact, previous treatments of the sample applied offer a recovery between 20% [19] or 40% [10] and is clearly improved before it can estimate the amount of food provided by melatonin. These recoveries can be affected by problems of stability of melatonin

This work aimed to provide evidence on how the quality of melatonin compound varies during the time under the influence of different environmental factors such as temperature, air and light, in order to establish the optimum storage conditions.

II. MATERIALS AND METHODS

A. Chemical reagents:

Melatonin standard was purchased from Sigma-Aldric™ (Somaprol, s.a.r.l). Methanol (HPLC grade) was purchased from Fluka, and glacial acetic acid for analysis was purchased from Riedel dehaën. Solutions were prepared by diluting with bidistilled water.

B. Melatonin sample preparation:

A 0.2 mg.L^{-1} of melatonin standard was prepared in bidistilled water. The calibration curve was linear over the following concentration ranges: 0.02 and 0.16 mg.$L^{-1}$of melatonin.

C. Storage conditions:

Table 1 shows the assayed storage conditions and codes used for them.

Table 1: Storage condition of melatonin and codes used to identify them.

Storage conditions	Temperatures (ºC)	Air	Light
D-32	-32	No	No
D4	4	No	No
D25	25	No	No
DA25	25	Yes	No
D50	50	No	No
L -32	-32	No	Yes
L4	4	No	Yes
L25	25	No	Yes
LA25	25	Yes	Yes
L50	50	No	Yes

Melatonin samples stored under the indicated conditions were analyzed by HPLC and after 2, 6, 8, 10, 13 and 15 days; comparing melatonin levels with initial levels of the same.

D. Determination and extraction of melatonin:

- *HPLC-FD*

Chromatographic analyses were carried out on an Alliance® System HPLC 2695 with pump system (Waters 600) and fluorescence detector .The column used in this study was Symmetry® C18 (5µm); 4,6mmx150mm from Waters. Millennium chromatographic software was used for HPLC control and peak integration.

An isocratic elution was used with two mobile phases: phase A (2% acetic acid and 8% methanol in water) and phase B (2% acetic acid and 8% water in methanol).Isocratic elution was used applying 50/50 (A: B) at a flow rate of 0.5 ml/min; and injection volume of 20µL. For the fluorescence detector; the fixed conditions were as follows: an excitation wavelength of **λ**= 280 nm and an emission wavelength of λ= 310 nm.

The HPLC mobile phases were first degassed in an ultrasonic bath and have filtered through a 0, 45 µm membrane before analysis with HPLC-FD.

III. RESULTS AND DISCUSSIONS

- *Storage stability*

Four variables that were thought likely to influence the stability of melatonin, such as temperature, light, air and time, were investigated.

In order to evaluate the effect of temperature, the results were compared to store solutions of melatonin in four different temperatures: -32ºC; 4ºC; 25ºC and 50ºC as is mentioned in table 1. Those solutions were analyzed for 15 days using different new vials for each control point.

The experimental data showing the effects of the storage conditions of melatonin standard are presented in **Fig. 1 & 2.**

Fig.1 shows the results of storage conditions of melatonin in light at five different storage locations: L -32; L4; L25; L50 and LA25.

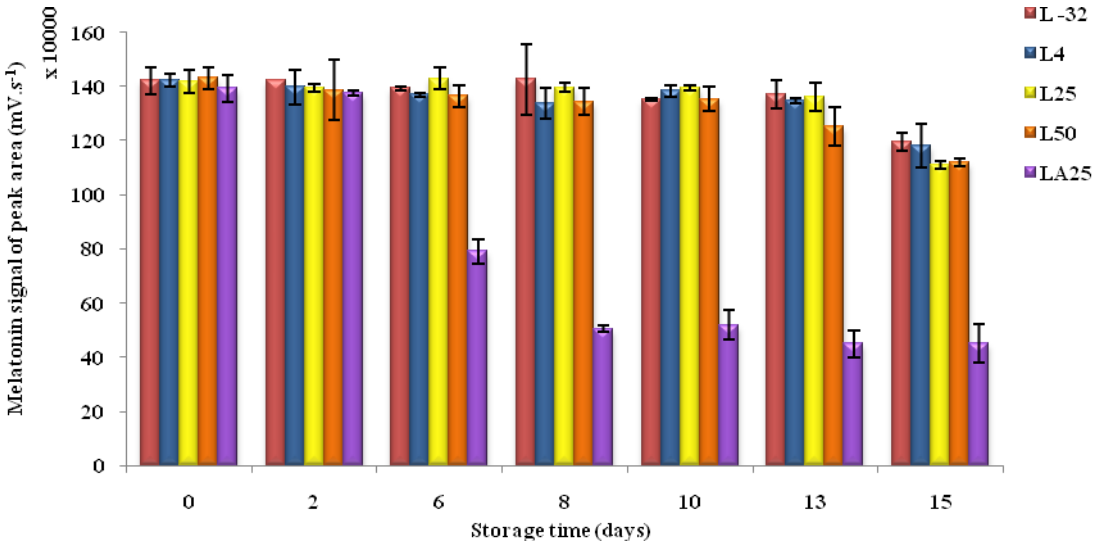

Figure 1: Time evolution of the samples stored in four different temperatures in the presence of light L-32, L4, L25 & L50ºC and in the presence of light with air at room temperature LA25ºC.

The results obtained demonstrate that the concentration of melatonin standard was not significantly decreased in all storage conditions until day 13, i.e. no significant degradation up to day 13. After this day, has been registered high degradation reaching up to 16% in L -32; 17 % in L4; 22% in L25 and 22% in L50. The samples kept at room temperature in the presence of light and air LA25, it was observed that the concentration of melatonin was significantly decreased start at sixth day of storage time reaching more and less 43% and suffers clear degradation on day 15 (68%). As it can be seen in **Fig. 1**, there are not significant

differences between results obtained using -32 ºC and 25 ºC at any point, therefore temperature is not

an important variable for melatonin degradation, at least using up to room temperature. Therefore, it can be concluded that melatonin solutions can be maintained up to thirteenth days between -32 and 25ºC without significant degradation.

Comparing all the samples stored in light only without air L-32, L4, L25, L50 with the samples stored in light and in contact with air LA25; we note that no differences between them and the samples remains stable until day 6. Regarding samples stored in light with air, the melatonin solutions showed melatonin degradation starting at day 6, then reaching up to 43%, more intense degradation was observed however on day 15 reaching up to 68%. Therefore light plus oxygen clearly increases the melatonin degradation. So, air is an important variable to be considered regarding melatonin stability. It has to be noted that no extra air was added to the samples vial through the storage, therefore it can be supposed that the initial fast degradation is due the air available at the beginning, then decreasing because no more air was added into the vial to maintain a longer stability of samples.

Thereafter, we have evaluated the effect of another variable which has been darkness, using the same five different storage locations. D -32; D4; D25; D50 and DA25. The results obtained are shown in **Fig. 2**.

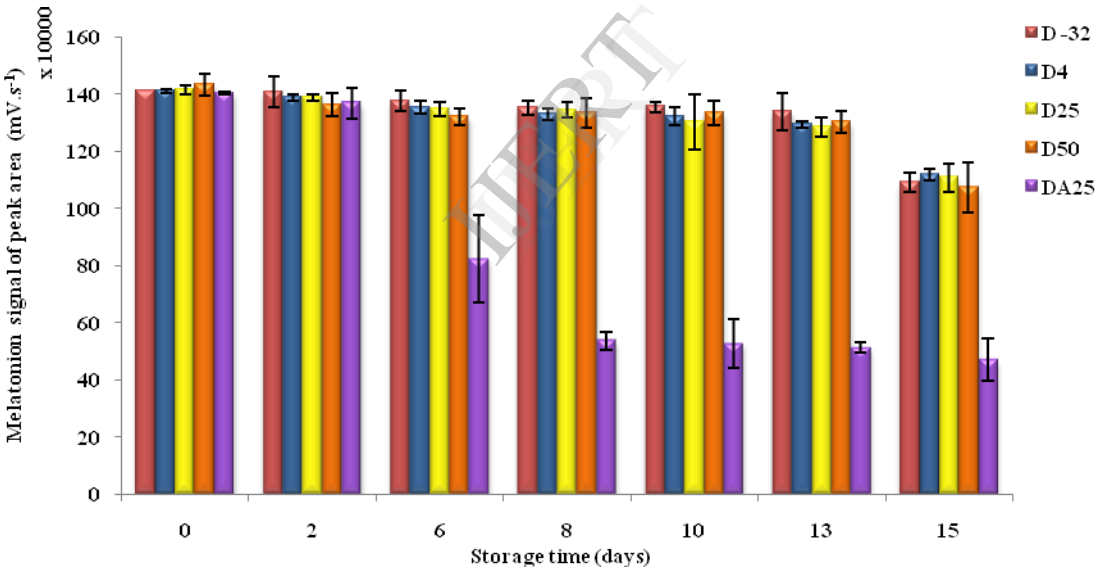

Figure 2: Time evolution of the samples stored in four different temperatures without light D-32, D4, D25 & D50ºC and without light & air at room temperature DA25ºC.

As it can be seen in **Fig. 2**, there are no differences between the results obtained for the samples stored in darkness without air at any point, and very similar results were obtained until day 13, except the samples stored at room temperature and in contact with air which start to degrade from the sixth day (41%) and more intense degradation was observed on day 15 (66%).

Comparing the storage conditions of samples stored in light presented in **Fig. 1** with the samples stored in darkness presented in **Fig. 2**, we note that there is no differences using light or light protected conditions at any control point and very similar results were obtained during storage time. Therefore, light is not an important variable to be considered regarding melatonin stability.

From the two Figures **1 & 2**, can be conclude that cannot be preserved melatonin samples both in darkness and in light and without air for more than 13 days, and in contact with air for more than 6 days.

IV. CONCLUSIONS

It has been shown that melatonin solutions are stable in storage conditions tested both in the presence of light or absence of it; except the samples preserved with air. Therefore the extracts can be stored using condition of air only for 6 days.

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