MELATONIN DOSAGE BY FIRST-DERIVATIVE SPECTROPHOTOMETRY

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Abstract. *The present work aims to develop a spectrophotometric method for simultaneous estimation of melatonin and Vitamin C in combined dosage form. Absorbance of melatonin and Vitamin C were measured at the absorbance maximum (λ_{max}) at 278 and 256 nm, respectively. The coefficient of correlation for melatonin at 278 nm and Vitamin C at 256 nm is 0.9986 and 0.9968, respectively. A linear relation between the absorbance and the melatonin concentration was found in the range 50-80 µg/ml, and a similar one (0-100 µg/ml for Vitamin C). The UV-Vis first derivative spectrophotometry method have been developed in this paper for the quantitative analysis of melatonin and Vitamin C in commercial capsules.*

Keywords: melatonin, first derivative spectrophotometry, vitamin C

1. INTRODUCTION

Melatonin is recognized as a potentially active constituent in many medicinal plants [1,2].

Melatonin (Fig.1) with the chemical formula: N-acetyl-3-(2-aminoethyl)-5-methoxyindole, is a neurohormone produced by the pineal gland, found in foods of plant origin, known as a biologically active compound, and with a potent anti-oxidative, anti-inflammatory and anticarcinogenic properties [3-6]. ML is an important component of the body's internal time keeping system and it is involved in important physiological events, as circadian rhythms (sleep wake cycle). Alterations in ML metabolism have been demonstrated in circadian rhythm sleep disorders, Alzheimer's and Parkinson's diseases, glaucoma, depressive disorder, breast and prostate cancer, hepatoma and melanoma [7].

As an antioxidant, melatonin generates some reactive oxygen and nitrogen species (superoxide ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), nitric oxide radical ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$)), many of them with therapeutic importance, blocking the peroxidation chain reaction caused by free radicals [8,9].

Spectrophotometric methods are widely used in the determination of different drugs [10], and also for melatonin, because this compund exhibits broad band ultraviolet and visible absorption bands. Several techniques have been reported in the literature for the determination of MEL individually in various biological samples such as HPLC, GC-MS, capillary electrophoresis, TLC, voltammetry, spectrophotometry, and fluorimetry [11-16].

The UV-Vis spectrophotometry method have been developed in this paper for the quantitative analysis of melatonin and Vitamin C in capsules dosage form and pure powder.

But, it has been used derivative spectrophotometry due to its capacity to separate shoulders and weak signals, improving the resolution of two or more analytical masked bands, and also, because is a selective analyse of drugs after simple dissolution of samples without complicated extraction procedures have been pointed out as an important advantage.

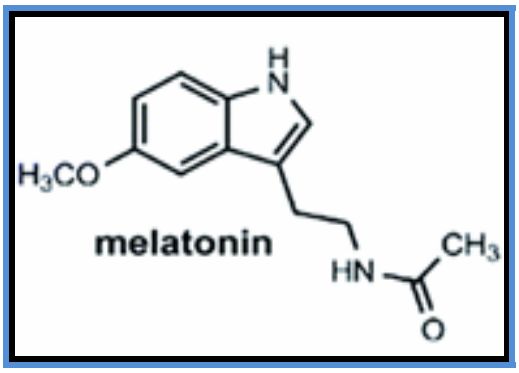

Fig. 1. The structure of melatonin

Derivative spectrophotometry (DS) is based on the mathematical calculation of the derivative values of the curve representing the absorption spectrum of a substance [17]. The UV-Vis spectrophotometry method have been developed in this paper for the quantitative analysis of melatonin and Vitamin C in capsules dosage form and pure powder.

2. EXPERIMENTAL SECTION

2.1. Materials

A fresh prepared stock solution of pure melatonin (Sigma Aldrich)(1 mg/ml) in etanol was properly diluted to obtain the samples (80 µg/ml) for the experiments. The spectra have been recorded in the wavelength range 200 and 500 nm, at the temperature 25 °C in all the experiments, just after preparation (t = 0) in order to avoid the possible degradation processes. Similar solutions have been prepared with Vitamin C, except the concentration range, which was 10-100 µg/ml. As comercial capsule has been used Geroprotect and for determinations, 20 capsules have been disolved in etanol and analyzed.

2.2. Characterization techniques

The samples were analyzed by using the following techniques:

The UV-Vis absorption spectra of the samples were recorded on a double beam M400 Carl Zeiss Jena UV-VIS spectrophotometer from 400 to 800 nm, at the resolution of 1 nm, with 1 nm slit width and 0.3 nm/s scan rate. The spectra have been obtained in 1cm matched quartz cells with the spectral bandwidth of 2 nm and wavelength accuracy of ±0.5 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (XB120A, Precisa, Switzerland).

3. RESULTS AND DISCUSSIONS

Spectrophotometric methods are widely used in the determination of several analytes and for the investigation of many analytical problems [18]. From standard stock solution of Melatonin different volumes were pipetted out in a series of seven 10 ml volumetric flasks, to obtain final solutions with the concentration between 50 and 80 µg/ml of drug. The absorbance of solutions containing melatonin at 80 µg/ml in etanol was spectrophotometrically measured in the UV range from 230 to 330 nm (Figure 2), showing a linearly dependence (Figure 3).

A calibration curve was constructed at optimum experimental conditions using absorbance versus concentration in the range of 50-80 µg/ml. Regression analysis using the method of least square was made for slope (0.028), intercept (0.0003) and correlation coefficient (0.9886) for λ = 278 nm and (0.9904) for λ = 309 nm. Similar results have been obtained for Vit C, λ = 256 nm and the correlation coefficient = 0.9998, Fig.4.

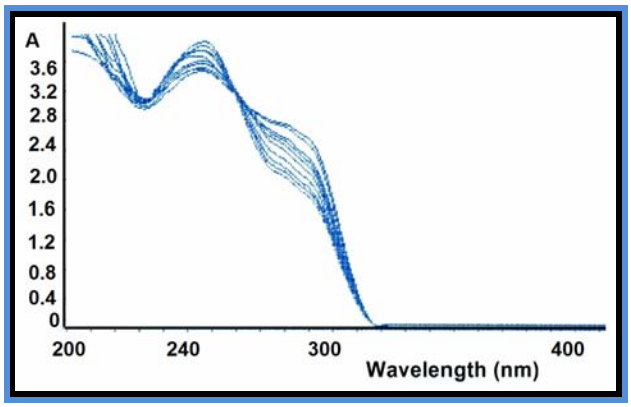

Fig.2. The absorption spectra variation of melatonin at different concentrations

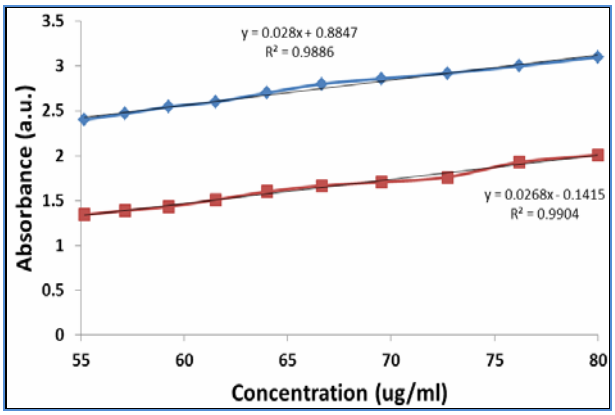

Fig.3. The linearity of melatonin absorbance at different concentrations

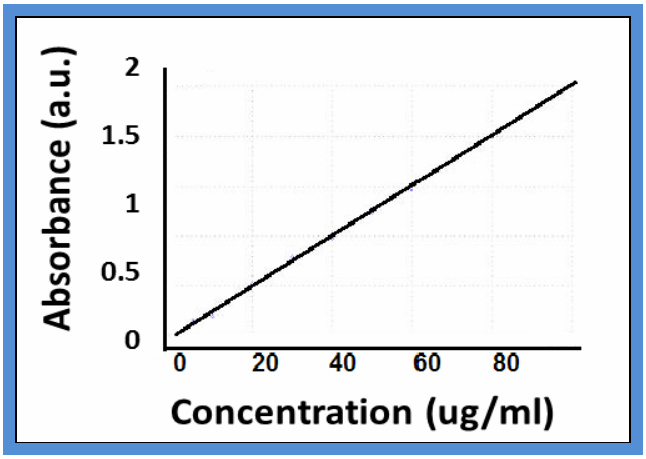

Fig. 4. The linearity of Vit C absorbance at different concentrations

The first-derivative spectra were obtained in the range of 200–400 nm, in arbitrary units as a function of the distance from the positive peak to the negative peak. Figure 5 shows the first-derivative spectra of solutions of pure melatonine in etanol, at different concentrations

varying from 80 µg/ml to 50 µg/ml, and the linearity of ^{1}D value is reprezented in Figure 6 for two wavelengths.

According to the data reported by Shida et al. [19], melatonine is soluble in a purely aqueous medium, and its concentration in this medium can be as high as 5 × 10^{-3} M.

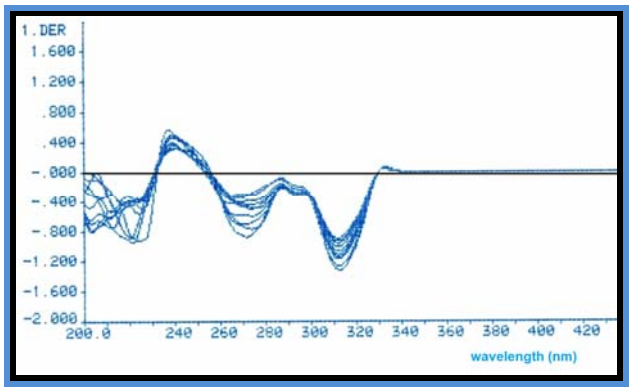

Fig.5. The ^{1}D spectra variation of melatonin at different concentrations

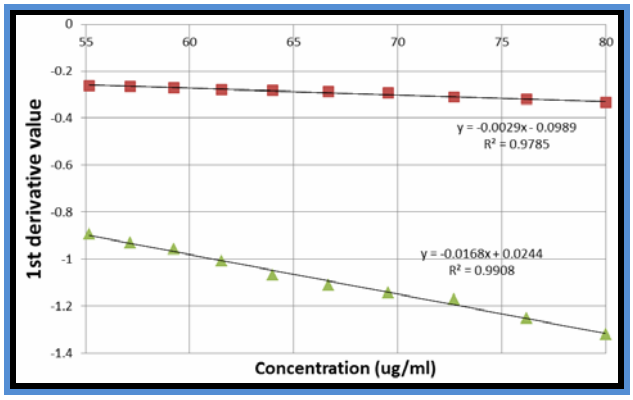

Fig. 6. Linearity of ^{1}D spectra versus concentration of melatonin in etanol solution.

Derivative spectrophotometry is an analytical technique of great utility for resolving some mixtures of compounds with overlapping spectra [20]. The coefficient of correlation for melatonin at 272 nm at 297 nm is 0.9785 and 0.9908, respectively.

Etanol solutions of Mel and Vit C were scanned separately in the range of 200-400 nm. These spectrums were converted to first derivative spectra by using derivative mode of apparatus. The two spectra were overlaid and it was observed that Mel showed zero crossing point at 231 nm, while Vit C showed ZCP at 250 nm respectively, these both wavelengths being selected as analytical wavelengths for determination of Mel and Vit C respectively.

The parameters for validating an analytical method for a solid pharmaceutical form was obtained. The linearity of the spectrophotometric response: a linear squared regression coefficient (r^2) of 0.99 that met the requirement established for the standards of quality.

By applying the above-mentioned data, we determined the amount of Melatonin and Vit C from Geroprotect, as follows: [Mel] = 320.7 µg/ml and [Vit C] = 679.3 µg/ml.

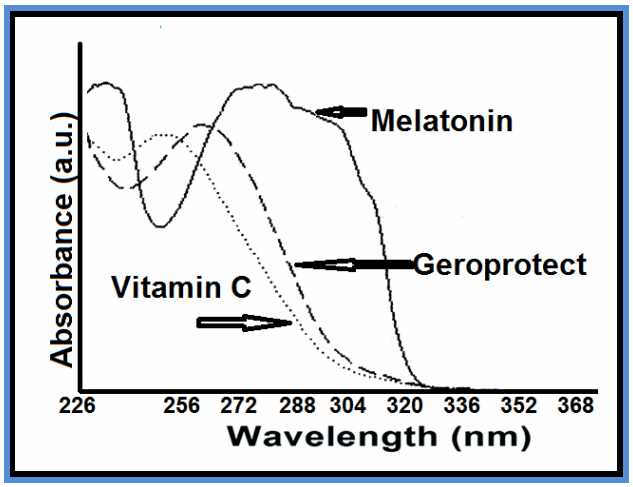

Fig.7. The absorption spectra of Mel, Vit C and GP

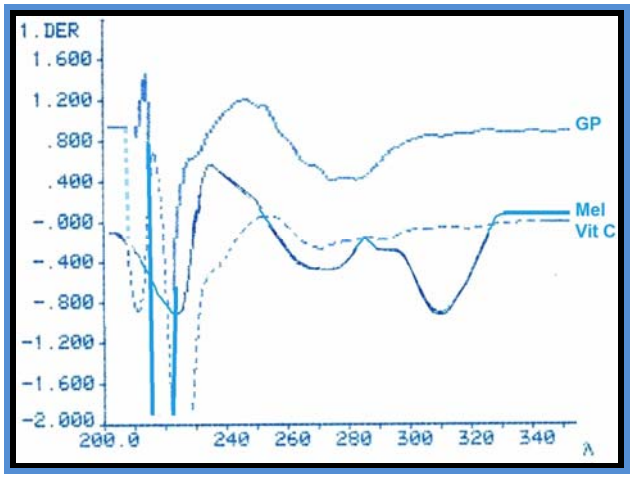

Fig.8. The ^{1}D spectra of Mel, Vit C and GP

4. CONCLUSIONS

This is the first study investigating the application of first derivative UV spectrophotometric method for Mel concentration evaluation alone and in mixture with Vitamin C in pharmaceutical capsule dosage forms.

Have been achieved absorption and the first derivative spectrophotometric spectra, and the calibration curves with regression coefficient for both of them. First order derivative spectrophotometric, is a selective, sensitive, rapid and can be used for the routine analysis and

quality control of Mel in its solid dosage formulations with Vit C.

5. ACKNOWLEDGEMENTS

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