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SIMULTANEOUS ESTIMATION OF MELATONIN AND HESPERIDIN BY UV SPECTROPHOTOMETRIC METHOD

K. B. Chougule*, M. B. Chougule, S. R. Devkar and P. M. Sangave

Womens College of Pharmacy, Peth – Vadgaon. Kolhapur, Maharashtra, India.

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***Corresponding Author**
K. B. Chougule
Womens College of Pharmacy, Peth – Vadgaon. Kolhapur, Maharashtra, India.

ABSTRACT

The present work aimed to develop and validate spectrophotometric methods for simultaneous estimation of melatonin and hesperidin, in combined dosage form. Method is based on solving a simultaneous equation. Absorbance of melatonin and hesperidin were measured at the respective absorbance maximum (λ max) at 274 and 298 nm. Methods are validated according to ICH guidelines. Linearity range for melatonin and hesperidin is 2-10 µg/ml and 2-10 µg/ml at respective selected wavelengths. The coefficient of correlation for melatonin at 274 nm and hesperidin at 298 nm is 0.997 and 0.993, respectively. A percentage estimation of melatonin and hesperidin from the liposome is 98.88% and 99.89% respectively, with standard deviation less than 2. The proposed method was simple, rapid, and validated and can be used successfully for the routine simultaneous estimation of melatonin and Hesperidin combined liposome formulation.

KEYWORDS: Melatonin, hesperidin, UV spectroscopic method, Simultaneous equation method, Method Validation, ICH guidelines.

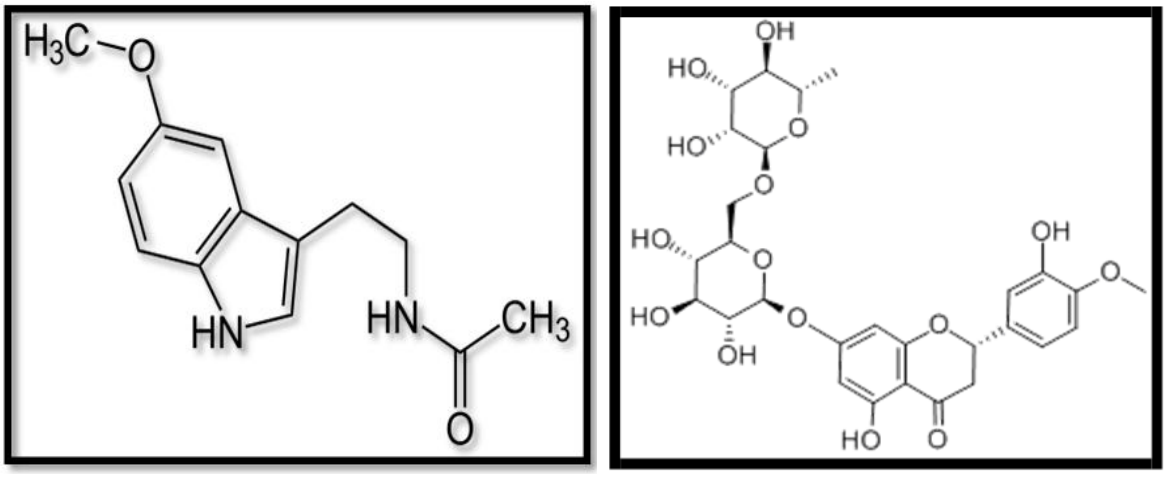
INTRODUCTION

Melatonin (MEL) chemically is an N – [2-5-methoxy -1H- indol-3-yl) ethyl] acetamide (figure 1 A), clinically used in the treatment of cancer, immune disorder, cardiovascular diseases, depression and sexual dysfunction. In animals, melatonin is secreted from the pineal gland during the night. It acts as a hormone, functioning as a circadian mediator for time information over the course of each day, and is also able to eliminate free radicals (reactive oxygen species). Melatonin also exists in higher plants (edible plants), and is inadvertently obtained from daily meals.[1,2] This substance was isolated by chance from the pineal gland, an endocrine organ, and is therefore named a hormone. Regarding the effect of melatonin in

inducing synchronization of circadian rhythms, which is generally regarded as a sleep-promoting effect, melatonin administration lowers deep body temperatures not only in those with rhythm disorders but also in healthy individuals, from children to elderly people[3]; shortens the time required to fall asleep; and improves sleep.[4] In addition, melatonin functions as an ant oxidative substance[5] and acts on bone metabolism.[6] Melatonin thus has a variety of activities. Melatonin is an amine of molecular weight 232 that is synthesized from tryptophan, an essential amino acid, via serotonin. It has been regarded as a specific hormone of the pineal gland, but is actually produced in the retina, brain (cerebral cortex, raphe nuclei, striate body, etc.), gastrointestinal tract (stomach, small intestine, etc.), testes, ovaries, spinal cord, lymphocytes, lens, cochlea, and skin. Melatonin is widely distributed not only in both vertebrate and invertebrate animals but also in plants such as rice, barley, and wheat.[1,8]

Hesperidine chemically know as (23)-5-hydroxy-2-(3-hydroxy-4 methoxy phenyl) 7 [(2S,3R,4S,5S,6R)-3,4,5 trihydroxy-6-{[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy 6 methyloxan -yl]oxymethyl] oxan-2-yl}oxy-2,3-dihydrochromen-4-one. This is act as antioxidant and anticonvulsant also.[9,10]

In the present study, an attempt has been made to develop a method for the simultaneous estimation of the melatonin and hesperidin. It can also be applied for routineanalysis of either one or any combinations of these drugs indosage forms. The method was validated in terms of stability, linearity, specificity, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ).



(A) **(B)**

Figure 1: Structure of (A) Melatonin and (B) Hesperidin.

MATERIAL AND METHODS

- **Material**

A double beam UV- spectrophotometer (Shimadzu UV -1800), volumetric flask and pipettes of borosilicate glass, bath sonicator (Labman 1-SL-50H) were used for development and validation of proposed analytical method.

Melatonin and Hesperidin dehydrate standard were purchased from Swapnroop drugs and pharmaceuticals, Aurangabad. Labmade liposome suspension, All the chemicals and reagent grade and were purchased from Molychem, Mumbai.

- **Method**

1. Preparation of Standard Stock Solutions

An accurately weighed quantity of MEL (10 mg) and HES (10 mg) were transferred to a separate 100 ml volumetric flasks, dissolved well and diluted to the mark with methanol to obtain standard solution having concentration of MEL (100 µg/ml) and HES (100 µg/ml). A 1 ml of both the solutions were transferred into a separate 10 ml volumetric flasks and diluted to the mark with methanol to obtain the solutions having the concentrations of 10 µg/ml for MEL and HES.

2. Methods (Calibration curve)

The standard solutions of MEL (10 µg/ml) and HES (10 µg/ml) were scanned separately in the UV range of 200-400 nm and the spectrum were recorded. The λ max values of MEL and HES were found to be 274 nm and 298 nm, respectively. From the standard stock solutions having concentrations2, 4, 6, 8 and 10 µg/ml for both MEL and HES were prepared in methanol. The absorbance of resulting solutions was measured at 274 nm and 298 nm and the calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using the calibration curve equations. The concentration of MEL and HES in the sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of MEL and HES at the selected wavelengths.

3. Method (Simultaneous Equation Method)

Two wavelengths selected for the method were 274 and 298 nm were the absorption maxima's of MEL and HES, respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series standard solution of 10 µg/ml.

The absorbances were measured at the selected wavelength and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determinations. Concentrations in the sample were obtained by using following equations.

CX = A1ay2-A2ay1/ A1ax2-A2ax1 CY= Ax1ay2-ax2ay1/ Ay1ax2-ay2ax1

Where A1 and A2 are absorbance's of mixture at 274 and 298 nm respectively, ax1 and ay1 absorptivities of MEL at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of HES at λ1 and λ2 respectively. Cx and Cy are concentration of MEL and HES respectively.

4. Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

- **Linearity (Calibration Curve)**

The calibration curves were plotted over a concentration range of 2-10 µg/ml for both MEL and HES (figure 3 & 4).

- **Method Precision (Repeatability)**

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for MEL and HES (10µg/ml for both MEL and HES) without changing the parameter of the proposed spectrophotometric method.

- **Intermediate Precision (Reproducibility)**

The intra-day and inter-day precision of the proposed method was determined by analyzing the sample solutions for three times on the same day and one time for three successive days.

- **Accuracy (Recovery study)**

The accuracy of the method was determined by calculating recovery of MEL and HES by the spiked method. To the sample solutions, known concentration of was added in different level viz., 80%, 100% and 120% level. The amounts of MEL and HES were recorded and calculated. This procedure was repeated for three times.

- **Limit of Detection and Limit of Quantification**

(LOD) and (LOQ) were calculated by constructing the calibration graph of MEL and HES at their selected wavelengths. LOD and LOQ were calculated from the slope and standard deviation of the response.

LOD=3.3×σ/s LOQ=10×σ/s

- **Analysis of MEL and HES in A liposome**

Multi-lamellar vesicles (MLV) liposomes consisting of mixtures of Phosphatidyl Choline and Cholesterol in different molar ratios) as lipid phase were obtained by thin film Hydration technique HES. Briefly, the lipid mixture and Melatonin and Hesperidin (1:4) was dissolved in 3:2 v/v of chloroform: methanol which was then removed under vacuum at 45°C, thus obtaining a thin film of dry lipid on the flask wall using a rotary flash evaporator until film was formed. After the dry residue appeared, to completely remove all the traces of solvent. The film was then hydrated by adding phosphate buffer (pH 5.4) under vigorous mechanical shaking with a vortex mixer until vesicle formation. The suspension was then centrifuged at 15000 rpm for 30-45 minutes and supernatant was decanted and pellet was dissolved in 10 ml methanol and sonicate. The above solution was suitably diluted with methanol to get final concentration of 10 µg/ml. The absorbance of liposome i.e. A1 and A2 were recorded at 274 nm and 298 nm and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equations generated by using absorptivity coefficients and absorbance values of MEL and HES at these selected wavelengths.

RESULTS AND DISCUSSION

UV Spectrophotometric method for simultaneous equation method was selected for the simultaneous estimation of MEL and HES. 274 nm (λ max of MEL) and 298 nm (λ max of HES) were selected as analytical wavelengths at which calibration curves prepared for both the drugs. (Figure 2). Linear correlation was obtained between absorbance and concentrations of MEL and HES in the concentration range 2µg/ml - 10µg/ml for both drugs. (Figure3 & 4) The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. LOD and LOQ values for MEL were found to be 0.55µg/ml and 1.69µg/ml at 274nm, respectively. LOD and LOQ values for HES were found to 1µg/ml and 3µg/ml at 298 nm, respectively. These data show that the method is sensitive for the determination of MEL and HES. The % RSD was found 0.20 and 0.12 for MEL and HES respectively. The proposed validated method was successfully applied to determine MEL and HES in their liposome suspension. The results obtained for MEL and HES were comparable with the corresponding labeled amounts (table 3). The relative standard derivation (% RSD) values for assay of MEL and HES were found to be and respectively. The %RSD was found to be less than 2%, which indicates that the proposed method is repeatable (table 4).

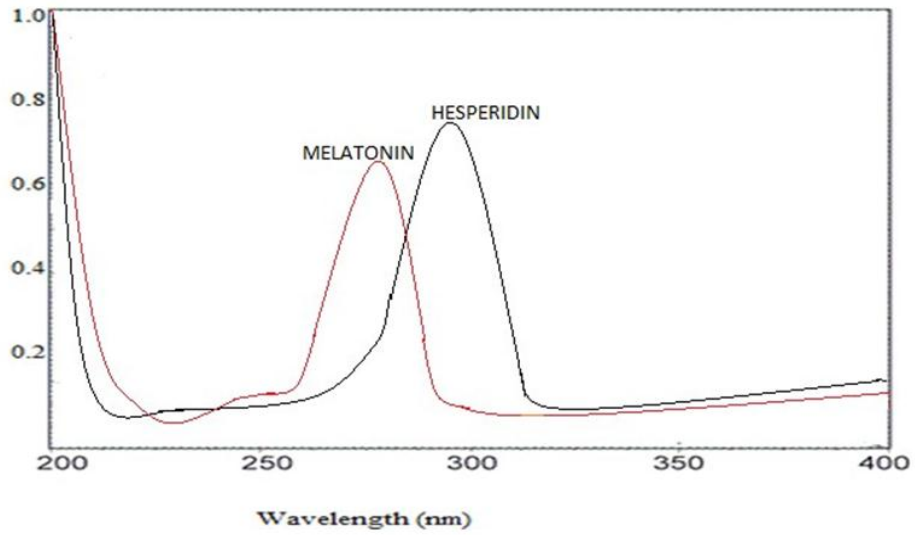


Figure No. 2: Overlay spectra of Melatonin and Hesperidin.

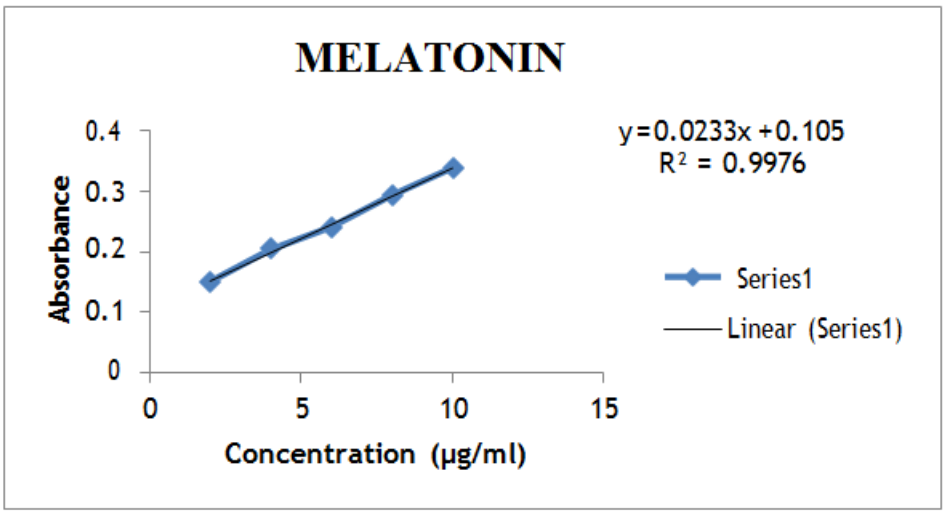


Figure No. 3: Linearity of Melatonin.

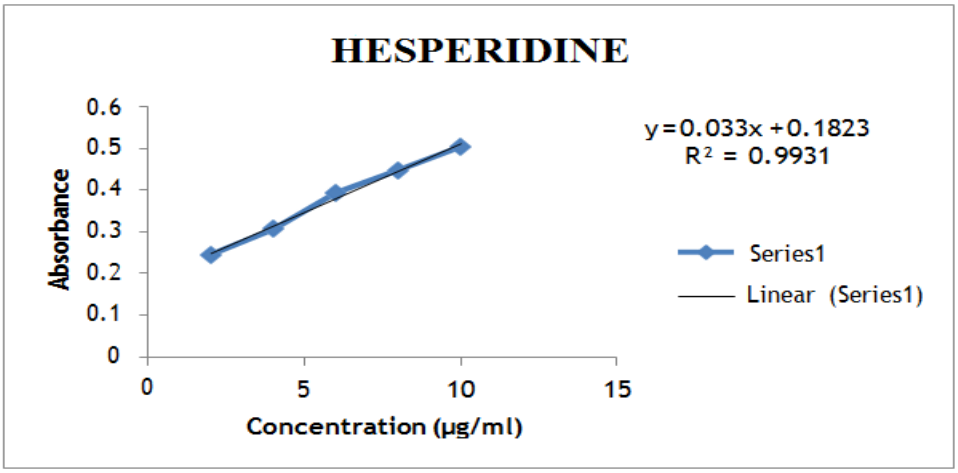


Figure 4: Linearity of Hesperidin.

Table 1: Regression analysis data and summary of validation parameter of the calibration curves.

Parameter	Melatonin	Hesperidin
Wavelength (nm)	274	298
Regression equation	0.023x+0.105	0.033x+0.182
Slop	0.023	0.033
Intercept	0.105	0.182
Correlation coefficient (R^2)	0.997	0.993
LOD(µg/ml)	0.55	1
LOQ(µg/ml)	1.69	3

Table 2: Result of recovery study.

Level of recovery	Amount of pure drug is added (µg/ml)		Amount of pure drug is found (µg/ml)		% recovery	
	MEL	HESR	MEL	HESR	MEL	HESR
80%	3.2	3.2	3.18	3.19	99.37	99.68
100%	4.00	4.00	3.98	3.99	99.5	99.75
120%	4.8	4.8	4.78	4.79	99.58	99.79

*Each value is mean of 3 determinations.

Table 3: Result of analysis of liposome suspension.

Drug	Label claim(mg)	Amount found	Percentage	SD	%RSD
Melatonin	1.8	1.78	98.88	0.0039	0.20
Hesperidin	9.2	9.19	99.89	0.0100	0.12

Table 4: Result of precision.

Day	% Label claim estimated			
	Melatonin	% RSD	Hesperidin	%RSD
Intra day	98.85	1.63	99.88	1.68
Inter day	98.80	1.57	99.87	1.58

CONCLUSION

The proposed method is applicable for the routine simultaneous estimation of MEL and HES in pharmaceutical dosage forms. The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for simultaneous determination of MEL and HES in liposome. The method utilizes easily available and low cost solvent like methanol for analysis of MEL and HES. Hence, the method was also found to be economical for the estimation of MEL and HES from liposome.

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