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First Order Derivative Spectrophotometric Method for Estimation of Melatonin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise and accurate method was developed for the estimation of melatonin in bulk and pharmaceutical dosage form using first order derivative spectrophotometry. Wavelength selected for quantitation was 219 nm for melatonin. The method was validated with respect to linearity, accuracy, precision, limit of detection and limit of quantitation in accordance with the International Conference on Harmonization (ICH) guidelines. Linearity was observed in concentration range of 2-12 µg/ml for melatonin. The limit of detection and limit of quantitation were found to be 10.60 µg/ml and 32.14 µg/ml for melatonin. The % R.S.D. values for intra-day and inter-day precision study were <1.0%, confirming that the method was sufficiently precise. The method can be successfully employed for the estimation of melatonin in pharmaceutical formulations.

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INTRODUCTION

Melatonin (N-acetyl-3-(2-aminoethyl)-5-methoxyindole), an endogenous hormone, is a predominant product of the pineal gland. It is secreted in a rhythm that is strictly dependent on the light–dark cycle. The melatonin plasma concentration rises in the early night, peaks at about midnight and then declines during the daytime [1]. Melatonin also has an age-related rhythm. The reduction of melatonin contributes to the aging process and may shorten the life span. Studies have shown that exogenous melatonin administration is useful for treating circadian disruptions, e.g. insomnia, jet lag. Moreover; melatonin has pharmacological effects on the treatment of Alzheimer's disease, Parkinson disease, glaucoma, depressive disorder, breast and prostate cancer, hepatoma and melanoma. Unfortunately, a substitution therapy is not easily achieved with melatonin because of its relatively poor bioavailability and rapid elimination.

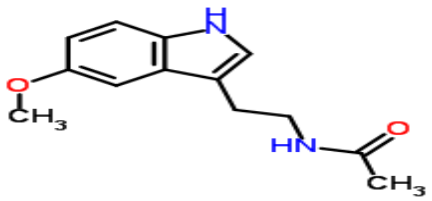

Fig. 1 Melatonin

In patients with sleep disorders and altered circadian rhythms, such as occur in jet lag, night shift work, and various neuropsychiatric disorders, oral administration of melatonin can provide the necessary resynchronization of those cycles, at dosages ranging from 0.3 to 8 mg. Synthesis of melatonin from the amino acid tryptophan is decreased by exposure to magnetic fields and by the aging process. Melatonin is a potent scavenger of free radicals and exerts direct inhibition of cancer growth. Various cancer types have been shown to be responsive to oral melatonin (10-50 mg daily), including breast cancer, non-small-cell lung cancer, metastatic renal cell carcinoma, hepatocellular carcinoma, and brain metastases [2] from solid tumors. Melatonin has also been reported to lower LDL- and total cholesterol levels. Abnormally low melatonin levels have been theorized to be a factor in multiple sclerosis, coronary heart disease, epilepsy, and postmenopausal osteoporosis. These reports, while preliminary, serve to further illustrate the wide range of potential effects exerted by melatonin [3,4,5]. It is soluble in water; soluble in methanol, Ethanol, slightly soluble in alcohol and in

chloroform; and very slightly soluble in acetone [6].

The present work aims at systematic development of a simple, rapid and highly sensitive method for the analysis of Melatonin by QbD approach.

MATERIALS AND METHODS

All chemicals used during the project work were either AR. The various reagents and chemicals used during experimental work are as follows;

Table 1: Chemicals and Instruments

Sr. No.	Name of Chemicals	Source	Sr. No.	Name of Equipment	Source
1.	Water	D/W	1.	UV	Shimadzu, Model: UV-1800
2.	Methanol	Analytical grade	2.	Electronic weighing balance	Shimadzu BL- 220 H
			3.	Sonicator	The ultrasonics PCi Analytics sonicator

Methods Preliminary solubility study of drug:

Solubility of the drug was determined at 28±1ºC. A small quantity of standard drug was dissolved in different solvents like distilled water, ethanol, methanol, acetonitrile, alcohol, chloroform, acetone.

Preparation of Stock solution: Preparation of standard stock solution of Melatonin:

10 mg of Melatonin accurately weighted by electronic balance and dissolved in 80 ml of double distilled water in 250ml conical flask. Content of flask was kept for stirring on magnetic stirrer for 10min and transferred in 100ml volumetric flask. Conical flask was rinsed by 20 ml of double distilled water and this water was used to make up volume 100 ml of volumetric flask to give conc. of 100µg/ml.

Preparation of working standard solution of Melatonin:

The working solution of Melatonin was prepared by further diluting the stock solution. Then pipette out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml and 1.2 ml of solution and make up to 10 ml

leads to 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml concentration solution. This solution was estimated by UV spectrophotometer by using Methanol as blank at 219 nm.

Fixing of wave length

After selecting the suitable solvent, the fixing of the λmax for the proposed method is very important. This can be done by scanning the drug sample (Melatonin) solution in Methanol in the range of 400nm-200 nm and the most repeated maximum absorbance with linearity and repeatability can be fixed as λmax for the drug. And in the proposed method for Melatonin drug shows maximum 219 nm. With more linearity, repeatability (ruggedness) and the λmax was fixed as 219 nm.

Linearity and range:

For linearity study from the working standard at different concentration 2, 4, 6, 8, 10 and 12 µg/ml of drug solution were placed in 6 different 10 ml volumetric flask volume was made up to the mark with Methanol. Absorbance was measured at 219 nm. Then obtained data were used for the linearity calibration plot.

Intra-day precision (repeatability) and inter-day precision study (intermediate precision): The standard stock solution of Melatonin was Prepared. Three concentration of (8, 10, and 12 µg/ml), were prepared by using mobile phase methanol. λmax was recorded at the intraday and inter day. % RSD was calculated. Variation of results within the day (intra-day), Variation of result between days (inter day) were analyzed. Intraday precision was analyzing Melatonin for three times in the same day at 219 nm. Inter-day precision was determined by analyzing the drug different day for three days at 219 nm. Precision data for Melatonin at 219 nm

Limit of Detection and Limit of Quantitation:

The limit of detection and quantification of drug are calculated with the standard deviation and slop.

$$LOD = \frac{3.3\sigma}{S} \quad LOQ = \frac{10\sigma}{S}$$

Where,

σ = standard deviation

S = slope of calibration curve

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2 to 12 mg/ml was linear with a correlation coefficient (R^2) 0.998.

Table 2: Linearity and range for melatonin at 219 nm

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.027
2.	4	0.030
3.	6	0.040
4.	8	0.052
5.	10	0.064
6.	12	0.073

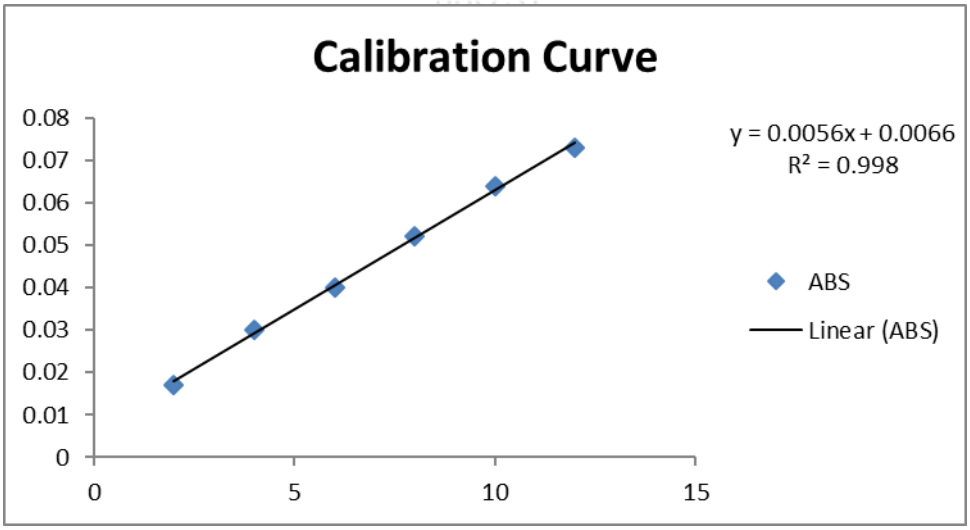

Figure 2: Linearity and range for melatonin

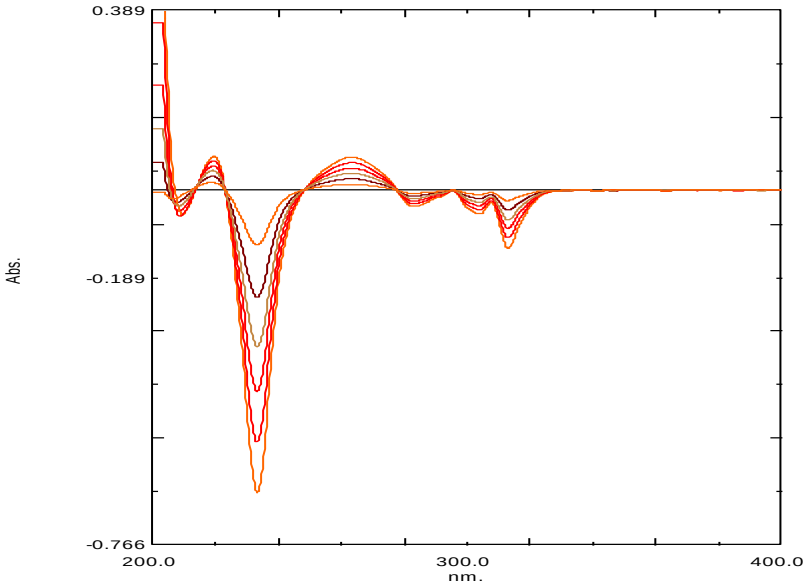

Figure 3: linearity sample overlay Melatonin

Table 3: Linearity Parameter

Parameter	Data
Range	2 µg/ml to 12 µg/ml
Correlation coefficient	0.998
Slope	0.0056
Intercept	0.0066

Intra-day precision (repeatability) and inter-day precision study (intermediate precision):

Table 4: Precision data for Melatonin 219 nm (Intra-Day)

Conc. (µg/ml)	Absorbance						AVG	SD	% RSD
8	0.058	0.058	0.056	0.057	0.057	0.057	0.057	0.00075	1.31
10	0.067	0.066	0.068	0.067	0.067	0.067	0.067	0.00063	0.94
12	0.072	0.071	0.071	0.071	0.072	0.073	0.071	0.00081	1.14

Table 5: Precision data for Melatonin at 219 nm (Inter-Day)

Conc. (µg/ml)	Absorbance						AVG	SD	% RSD
8	0.049	0.048	0.048	0.049	0.048	0.048	0.048	0.00051	1.06
10	0.060	0.061	0.061	0.061	0.060	0.060	0.060	0.00054	0.9
12	0.068	0.068	0.057	0.069	0.069	0.070	0.066	0.0048	1.2

Limit of Detection and Limit of Quantitation:

$$LOD = \frac{3.3\sigma}{S} \quad LOQ = \frac{10\sigma}{S}$$

Where,

σ = standard deviation

S = slope of calibration curve

Table 6: Limit of Detection and Limit of Quantitation

LOD	LOQ
10.60	32.14

RESULTS AND DISCUSSION

Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between absorbance and concentration over the ranges 2-12 µg/ml for melatonin. The linear equations for the calibration plots were y = 0.0056x+0.066 with Regression (r^2) being 0.998 for melatonin.

Precision

The precision of the method was expressed as relative standard deviation (RSD %).The % R.S.D. values for intra - day precision study and inter - day study listed in (Table 4 and 5) were<1.0%, confirming that the method was sufficiently precise.

LOD and LOQ

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 10.60 µg/ml and 32.14 µg/ml for melatonin

CONCLUSION

The proposed methods were found to be accurate, precise, and economical and can be applied for routine quality control analysis of Melatonin in pharmaceutical dosage form. Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money.

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