A Simple UV Spectrophotometric Method Development and Validation of Melatonin in its Bulk and Pharmaceutical Dosage Forms

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Abstract: A simple and sensitive UV Spectrophotometric method has been developed for the quantitative estimation of Melatonin in its bulk and pharmaceutical dosage form. The drug showed maximum absorbance at 225nm. Regression analysis of Beer's plot showed correlation of 0.998 in the concentration range of 1.5-7.5 µg/ml and the percentage recovery was between 98-102% indicting high degree of accuracy. The % RSD of intra-day and inter-day precision was found to be less than 2 as per ICH guidelines. Sensitivity of the method was confirmed by accurate LOD and LOQ values which were obtained 0.23 and 0.698 respectively.

Key words: Melatonin, Spectroscopic method development, validation, ICH Guidelines.

INTRODUCTION

UV Visible Spectroscopy:

The UV-visible range is only a small part of the total electromagnetic spectrum, and is generally defined from wavelength of 190nm at the high energy UV end to about 750nm at the low energy red end of the spectrum. In ultraviolet/visible spectroscopy, a molecule absorbs ultraviolet light, leading an electron to be promoted from a ground electronic state to an excited electronic state. This absorption spectroscopy divides the electromagnetic spectrum into the ultraviolet (UV, 190-400nm) and visible (VIS, 400-800nm) sections.

Ultraviolet light: wavelengths between 190 and 400 nm Visible light: wavelengths between 400 and 800 nm [1-9]

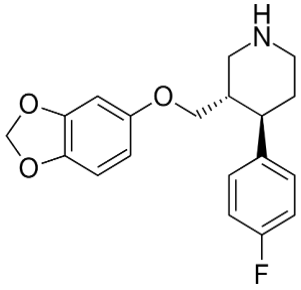
Drug Profile:

Melatonin is a hormone produced by the pineal gland that has multiple effects including somnolence, and is believed to play a role in regulation of the sleep-wake cycle. Melatonin is available over-the-counter and is reported to have beneficial effects on wellbeing and sleep. Melatonin has not been implicated in causing serum enzyme elevations or clinically apparent liver injury. [10]

Category	Circadian rhythm sleep disorders
I.U.P.A.C Name	N- [2-(5-methoxy-1H-in dol-3-yl) ethyl] acetamide
Description	White powder.
Chemical Formula	$C_{13}H_{16}N_2O_2$
Molecular Mass	433.4947 g/ mol.
Storage	Store at room temperature. Don't refrigerate.
Maximum Wavelength (λ_{max})	225nm (UV)
Brand Name	MELOKALM
Solubility	Soluble in distilled water, 0.1N NaOH, 0.1N HCl, Dimethyl sulphoxide, Ethanol, Methanol.

Table 1: Drug Profile of Melatonin

Fig 1: Structure of Melatonin



There are many methods available in literature till date pertaining to method development and validation of Melatonin by UV Spectrophotometric, Colorimetric, HPLC, UPLC, LS-MS. Most of the work has been carried out in combination with other drugs. The one which is available as single drug was carried out using Buffer as solvent. [11-29]

Method And Materials:

PREPARATION OF STOCK SOLUTION:

10mg of the API is accurately weighed and transferred into clean 10ml volumetric flask and solubilized in 1ml of methanol and make it up to 10ml by using distilled water which gives us the concentration of 1000µg/ml.

PREPARATION of 100µg/ml:
From the above stock solution 1ml is transferred into a 1ml volumetric flask and made up to 10ml with distilled water which gives us the concentration of 100µg/ml.
PREPARATION SERIAL DILUTIONS:
From the above solution 1.5,3,4.5,6,7.5 transferred into a 10 ml volumetric flask and made up to 10ml with distilled water which gives us the concentration of 1.5,3,4.5,6,7.5µg/ml.
Assay:
Twenty tablets of 'Health Vit Melatonin' (West Coast Pharmaceuticals) were weighed and grounded to a fine powder. Weigh and transfer the powder equivalent to 10 mg of tablet powder into a 100 mL volumetric flask, add 20 mL of methanol and sonicate for 30 min, further make up the volume with diluent. A portion of this solution was filtered through a 0.22 µm membrane filter (discarding the first few mL of the filtrate).
From the filtered solution 5 mL was pipetted out into a 50 mL volumetric flask and made up to 50 mL with diluent. This solution (1µL) was checked for absorbance for three times. The mean absorbance and its content in the formulation was calculated.

Formulation	Label claim mg/tab	Amount found Mean ± S. D	Assay	%RSD
Tablet	10	99.89 ± 0.0054	99.89	0.0054

Table 2: Assay Results

RESULTS AND DISCUSSION

SPECIFICITY:
The analyte was assessed in the presence of components and does not show any interaction.
LINEARITY:
Various standards in the range 1.5-7.5µg/ml of Melatonin was observed in to UV system. A graph of absorbance (on Y-axis) versus concentration (on X-axis) is plotted and the correlation coefficient was calculated.

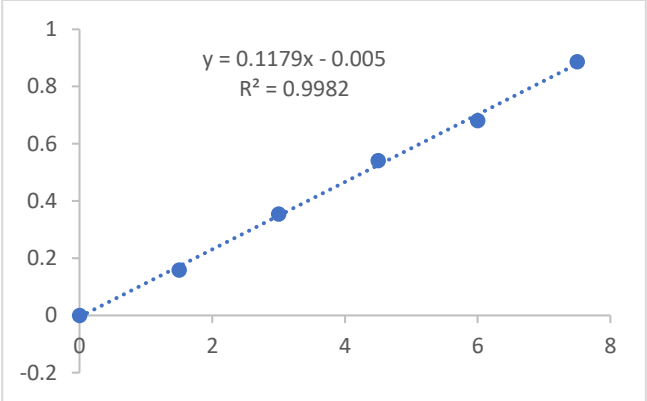


Figure 2: Calibration Curve

Concentration (µg/ml)	Absorbance
1.5	0.159
3	0.354
4.5	0.541
6	0.681
7.5	0.887

Table 3: Linearity values

ACCURACY:

S. no	Level of addition	Amount of Pure Drug (ml)	Absorbance	Amount of Drug found	% Recovery
1	50	0.225	0.798	6.7286	99.6
2	50	0.225	0.799	6.7288	99.7
3	50	0.225	0.798	6.7289	99.6
4	100	0.45	1.065	8.9934	99.8
5	100	0.45	1.066	8.9935	99.9
6	100	0.45	1.065	8.9933	99.9
7	150	0.675	1.345	11.362	101.6
8	150	0.675	1.344	11.361	101.6
9	150	0.675	1.283	11.363	101.7

Table 4: Accuracy Values

PRECISION:
INTRA-DAY PRECISION:

S.no	Concentration (µg/ml)	Absorbance
1	4.5	0.541
2	4.5	0.54
3	4.5	0.541
4	4.5	0.541
65	4.5	0.542
6	4.5	0.541
MEAN		0.541
SD		0.000632
%RSD		0.116905

Table 5: Intraday Precision

INTER DAY PRECISION:

S.no	Concentration (µg/ml)	Day -1	Day -2

1	4.5	0.541	0.540
2	4.5	0.542	0.539
3	4.5	0.541	0.540
4	4.5	0.542	0.543
5	4.5	0.541	0.545
6	4.5	0.541	0.541
	MEAN	0.411833	0.4115
	STD	0.001169	0.000548
	%RSD	0.283864	0.133104

Table 6: Interday Precision

ROBUSTNES:

S.no	Concentration (µg/ml)	ABSORBANCE	
		220nm	230nm
1	4.5	0.541	0.544
2	4.5	0.542	0544
3	4.5	0.541	0.546
MEAN		0.541333	0.544333
STD		0.000577	0.001528
%RSD		0.106653	0.28062

Table 7: Robustness

RUGGEDNESS:

S.no	Concentration (µg/ml)	Analysist -1	Analysist - 2
1	4.5	0.541	0.540
2	4.5	0.542	0.539
3	4.5	0.541	0.540
	MEAN	0.541667	0.543
	STD	0.000577	0.001
	%RSD	0.106588	0.184162

Table 8: Ruggedness

Limit of detection:

LOD = 3.3*S. D/SLOPE
=3.3*1.146/0.1179
=0.23

Limit of quantification:

LOQ =10*S. D/SLOPE
=10*1.146/0.1179
=0.698

CONCLUSION

The %RSD values were within the limits and the method was found to be precise. The results expressed in UV method were promising. The UV method is more sensitive, accurate and precise. This method can be used for the routine determination of melatonin in bulk drug and in pharmaceutical dosage forms. Degradation studies has also been studied. Method development and validation is followed according to the ICH guidelines and the all the parameters were validates and found to be within the limits.

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