ISSN No: - 2456 – 2165

Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Melatonin and Quercetin in Liposome Formulation

V.C.Yeligar, M.A.Rajmane*, K.B.Chougule, V.K.Chougule,S.S.Patil
Department of Quality Assurance Techniques,
Ashokrao Mane College of Pharmacy, Peth – Vadgaon,
Kolhapur, Maharashtra, India 416112

Abstract :- The present work aimed to develop and validate spectrophotometric methods for simultaneous estimation of melatonin and quercetin in combined dosage form. Method is based on solving a simultaneous equation. Absorbance of melatonin and quercetin were measured at the respective absorbance maximum (λ max) at 276 and 372 nm. Methods are validated according to ICH guidelines. Linearity range for melatonin and quercetin is 2-10 µg/ml and 2-10 µg/ml at respective selected wavelengths. The coefficient of correlation for melatonin at 276 nm and quercetin at 372 nm is 0.997 and 0.993, respectively. A percentage estimation of melatonin and quercetin from the liposome is 99.5 % and 99.75 % 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 quercetincombined liposome formulation.

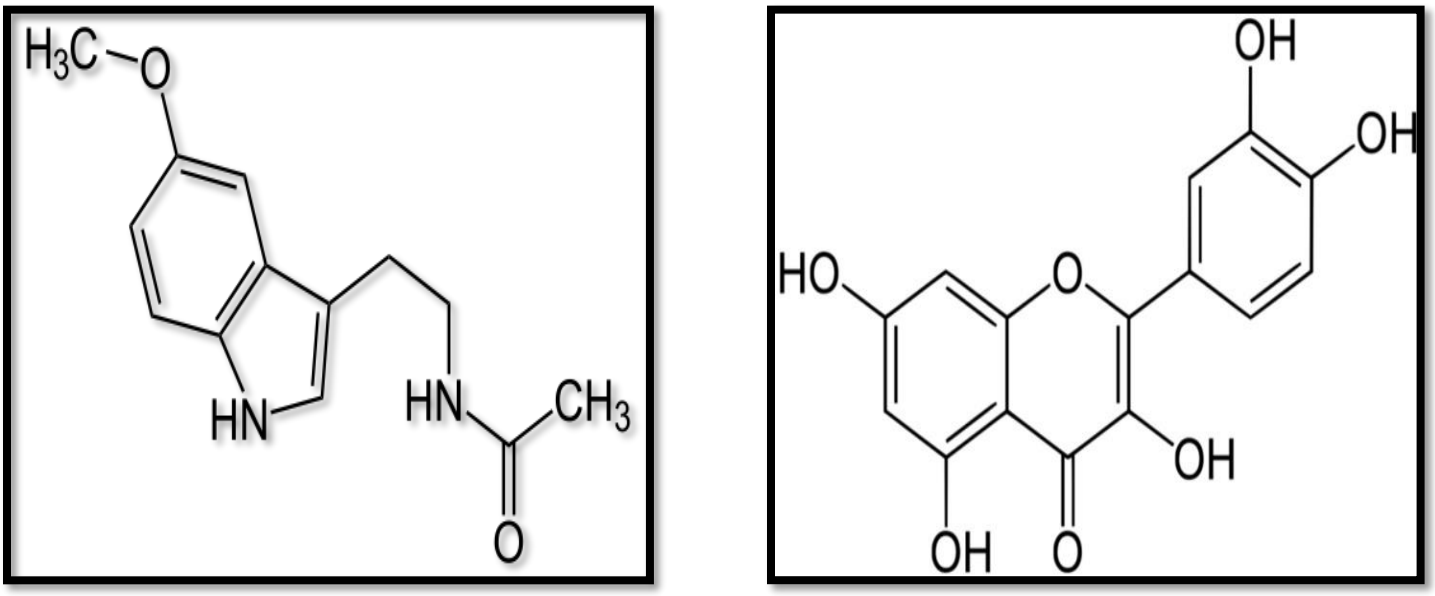
Keywords:- *Melatonin, quercetin, UV spectroscopic method, Simultaneous equation method, Method Validation, ICH guidelines.*

I. 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 antioxidative 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].

Quercetin is chemically, 2-(3,4-dihydroxy phenyl0-3,5,7-trihydroxy-4H-chromen-4-on.(figure 1 B) Quercetin, a flavonols, is a plant derived flavonoid found in fruits, vegetables, leavesand grains. It also may be used as an ingredient of supplements, beverages or foods. Quercetin is aflavonoid widely distributed in nature. Quercetin is a flavonoid widely distributed in nature. Quercetin is frequenly used therapeutically in allergic conditions, including asthma, hay- fever, eczema and hives. Additional clinical uses include treatment of gout, pancreatitis and prostatitis; also used in inflammatory conditions. Quercetin is used for treating conditions of the heart and blood vessels including “hardening of the arteries.”(atherosclerosis), high cholesterol, and heart disease and circulation problems. It is also used for diabetics, cataract, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, chronic fatigue syndrome and preventing cancer and for treating chronic infection of the prostate.[4,5,6]

ISSN No: - 2456 – 2165



(A) (B)

Figure 1 Structure of (A) Melatonin and (B) Quercetin

II. 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 Quercetin 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 QUE (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 QUE (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 QUE.

2. Methods (Calibration curve)

The standard solutions of MEL (10 µg/ml) and QUE (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 QUE were found to be 276 nm and 372 nm, respectively. From the standard stock solutions having concentrations2,4,6,8 and 10 µg/ml for both MEL and QUE were prepared in methanol. The absorbance of resulting solutions was measured at 276 nm and 372 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 QUE in the sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of MEL and QUE at the selected wavelengths.

3. Method (Simultaneous Equation Method)

Two wavelengths selected for the method were 276 and 372 nm were the absorption maxima's of MEL and QUE, 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 absorbance's 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 276 and 372 nm respectively, ax1 and ay1 absorptivities of MEL at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of QUE at

ISSN No: - 2456 – 2165

λ1 and λ2 respectively. Cx and Cy are concentration of MEL and QUE 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 QUE (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 QUE (10µg/ml for both MEL and QUE) 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 QUE by the spiked method. To the sample solutions, known concentration of was added in different level viz., 80,100 and 150% level. The amounts of MEL and QUE 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 QUE at their selected wavelengths. LOD and LOQ were calculated from the slope and standard deviation of the response.

$LOD=3.3\times\sigma/s$

$LOQ=10\times\sigma/s$

- **Analysis of MEL and QUE 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. Briefly, the lipid mixture and Melatonin and Quercetin (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 decanded 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 276 nm and 372 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 QUE at these selected wavelengths.

III. RESULTS AND DISCUSSION

UV Spectrophotometric method for simultaneous equation method was selected for the simultaneous estimation of MEL and QUE. 276 nm (λ max of MEL) and 372 nm (λ max of QUE) were selected as analytical wavelengths at which calibration curves prepared for both the drugs. (Figure 2). Linear correlation was obtained between absorbances and concentrations of MEL and QUE in the concentration range 2-10 µg/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 0.57µg/ml and 1.71µg/ml at 276nm, respectively. LOD and LOQ values for Que were found to 1µg/ml and 3µg/ml at 372 nm, respectively. These data show that the method is sensitive for the determination of MEL and QUE. All the regression analysis data and the summary of validation parameters for the proposed method.The% RSD were0.21 and 0.12 for MEL and QUE, respectively, The proposed validated method was successfully applied to determine MEL and QUEin their suspension. The results obtained for MEL and QUE were comparable with the corresponding labeled amounts (table 3). The relative standard derivation (% RSD) values for assay of MEL and QUE 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).

ISSN No: - 2456 – 2165

Figure No.2: Overlay spectra of Melatonin and Quercetin

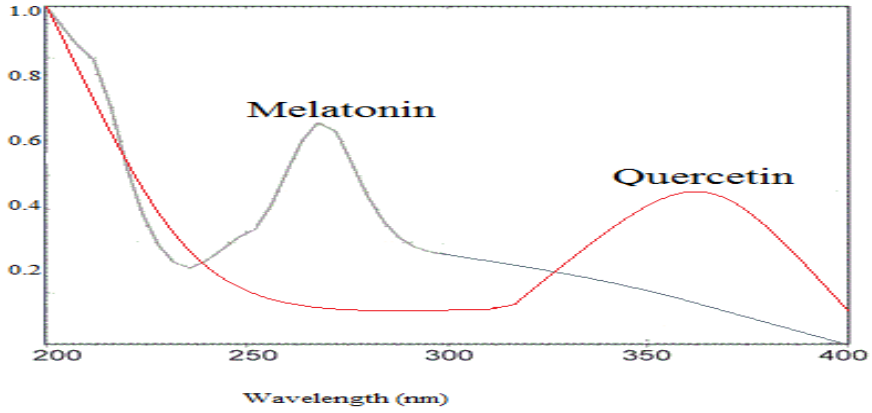


Figure No.3: Linearity of Melatonin

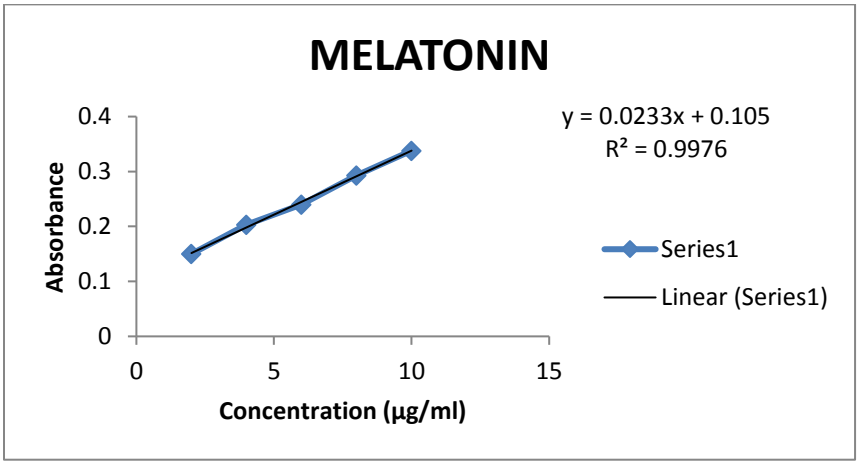
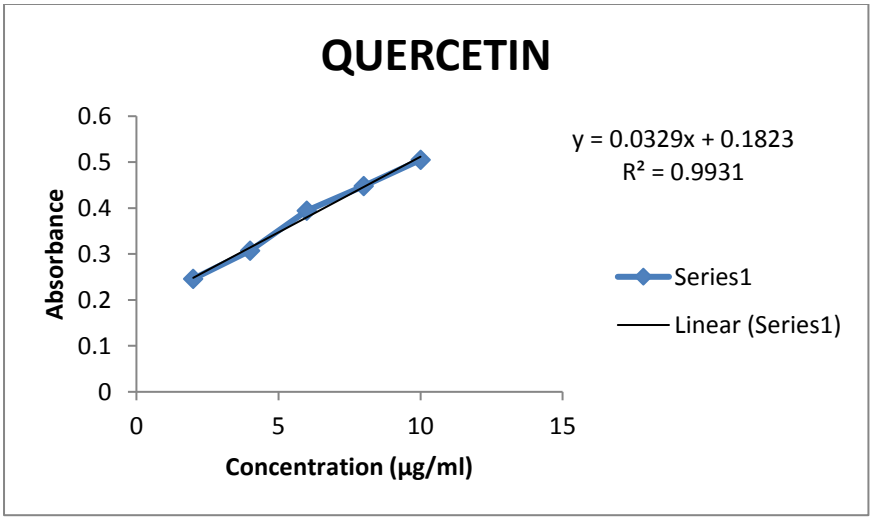


Figure: 4 Linearity of Quercetin



ISSN No: - 2456 – 2165

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

Parameter	Melatonin	Quercetin
Wavelength (nm)	276	372
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)	0.57	1
LOQ(µg/ml)	1.71	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	**QUER**	**MEL**	**QUER**	**MEL**	**QUER**
80 %	3.2	3.2	3.15	3.19	98.43	99.68
100 %	3.4	3.4	3.38	3.37	99.41	99.11
150 %	3.6	3.6	3.59	3.58	99.72	99.44

*Each value is mean of 3 determinations.

ISSN No: - 2456 – 2165

Table 3: Result of analysis of liposome suspension

Drug	Label claim(mg)	Amount found	Percentage	SD	%RSD
Melatonin	2	1.99	99.5	0.004	0.21
Quercetin	8	7.98	99.75	0.010	0.12

Table 4: Result of precision

Day	% Label claim estimated			
	Melatonin	% RSD	Quercetin	%RSD
Intra day	99.66	1.65	99.68	1.68
Inter day	99.70	1.59	99.65	1.58

IV. CONCLUSION

No interference of the excipients with the absorbance of interest appeared, hence the proposed method is applicable for the routine simultaneous estimation of MEL and QUE in pharmaceutical tablet dosage forms. The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for simultaneous determination of MEL and QUE in liposome. The method utilizes easily available and low cost solvent like methanol for analysis of MEL and QUE. Hence, the method was also found to be economical for the estimation of MEL and QUE from liposome.

REFERENCES

[1]. Hattori A., Migitaka H., Iigo M., et al "Identification of melatonin in plants and its effects on plasma melatonin levels and binding tomelatonin receptors in vertebrates."BiochemMolBioInt 35:627-634(1995)

[2]. Oba S., Nakamura K., Sahashi Y., Hattori A., Nagata C."Consumption of vegetables alters morning urinary 6-sulfatoxymelatonin concentration". J Pineal Res 45: 17-23(2008)**.**

[3]. Krauchi K, Cajochen C, Wirz-Justice A. "A relationship between heat loss and sleepiness: effects of postural change and melatonin administration."JApplPhysiol 83: 134-139 (1997)**.**

[4]. McArthur A.J., Lewy A.J., Sack R.L."Non-24-hour sleep-wake syndrome in a sighted man: circadian rhythm studies and efficacy of melatonin treatment Sleep" 19: 544-553(1996)

[5]. Reiter R.J., Tan D.X., Osuna C., Gitto E."Actions of melatonin in the reduction of oxidative stress. A review." J Biomed Sci 7: 444-458(2000)

[6]. Suzuki N., Somei M., Seki A.,Reiter R., Hattori A."Novel bromomelatonin derivatives as potentially effective drugs to treat bone diseases." J Pineal Res 45: 229-234(2008)

[7]. Raskind M.A., Burke B.L., Crites N.J.,Tapp A.M., Rasmussen D.D."Olanzapine-induced weight gain and increased visceral adiposity is blocked by melatonin replacement therapy in rats."Neuropsychopharmacology 32: 284-288(2007)

[8]. Paredes S.D., Korkmaz A., "Manchester L.C., et al: Phytomelatonin: a review." J Exp Bot 60: 57-69(2009).

[9]. Hassan N.A., Barrsoum B.N., Habib I.H.I., "Simultaneous spectrophotometric determination of rutin, quercetin and ascorbic acid in drugs using a Kalman Filter approach". Journal of Pharmaceutical and Biomedical Analysis,20: 315-320(1999)**.**

[10]. Irena Baranowska, DominikaRaro' g."Application of derivative spectrophotometry to determination of flavonoid mixtures, Talanta" 200(55):209-212**..**

[11]. Pejic N., Kuntic V., Vujic Z., Micic S., "Direct spectrophotometric determination of quercetin in the presence of ascorbic acid". IL FARMACO.59: 21-24(2004).