Method development and validation for simultaneous estimation of zolpidem and melatonin by RP-HPLC in tablet dosage form

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Received on: 17-02-2012; Revised on: 12-03-2012; Accepted on: 22-04-2012

ABSTRACT

A rapid high performance liquid chromatographic method has been developed and validated for the estimation of zolpidem and melatonin simultaneously in combined dosage form drug was resolved on a c18 column (LENGTH 250mm*DIAMETER 4.6mm i.d. Particle size 5µm) in isocratic mode. The mobile phase used was acetonitrile and phosphate buffer in the ratio 40:60 mobile phase was delivered at the flow rate of 1ml/min. Ultraviolet detection was carried out at 231nm. Calibration curve was linear with correlation coefficient \( r^2 \) =0.999. The selected chromatographic conditions were found to separate zolpidem (rt= 3.66 ) and meltonin (rt= 2.51 ) having a resolution of 7.416min. The proposed method can be used for the analysis of commercially available dosage form (zolpidem tartarate and melatonin) in combined dosage form.

Keywords: Zolpidem Tartrate, Melatonin, High performance liquid chromatography, C18 column, RP-HPLC

INTRODUCTION:
Zolpidem Tartrate is chemically \( \text{N,N,6-trimethyl-2-p-tolylimidazo[1,2-a] pyridine-3-acetamide L-(+)-tartrate} \) (figure 1). It is short-acting non benzodiazepine hypnotic of the imidazopyridine class that potentiates gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, by binding to GABA receptors at the same location as benzodiazepines.1-3

Melatonin also known chemically as \( \text{N}-\text{acetyl-5-methoxytryptamine} \) (figure 2) is a naturally occurring compound found in animals, plants, and microbes. It has anticonvulsants activity, CNS depressant activity. Melatonin is a derivative of tryptophan. It binds to melatonin receptor type 1A, which then acts on adenylate cyclase and the inhibition of a cAMP signal transduction pathway. Melatonin not only inhibits adenylate cyclase, but it also activates phosphilpase C. This potentiates the release of arachidonate. By binding to melatonin receptors 1 and 2, the downstream signalling cascades have various effects in the body. The melatonin receptors are G protein-coupled receptors and are expressed in various tissues of the body. There are two subtypes of the receptor in humans, melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2). Melatonin and melatonin receptor agonists, on market or in clinical trials, all bind to and activate both receptor types.

Zolpidem tartarate and melatonin combination tablet dosage forms are available in tablet dosage forms in the ratio of 6:10. Literature survey reveals, UV, HPLC methods for analysis melatonin of single and combined dosage forms with other drugs and UV, HPLC, LCESI MS/MS methods for analysis Zolpidem Tartrate of single and combined dosage forms with other drugs. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines10-11.

MATERIALS AND METHODS:

Instrument:
The HPLC system used was Shimadzu with model prominence equipped with UV detector source of deuterium lamp. The chromatogram was recorded at and peaks quantified by means of PC based Spinchrome software.

Reagents and Chemicals:
Acetonitrile (AR Grade) and phosphate buffer were used as solvent. Hplc grade water and Pure Standard gift sample of zolpidem tartarate and melatonin provided by Chandra labs, Hyderabad. Tablets of zolsoma Fc Tabs (zolpedem 5mg and melatonin 3mg ) were purchased from local market.

CHROMATOGRAPHIC CONDITIONS:

Model : Prominance
Software : Spinchrom 21CFR
Column : C18,( 250nm*4.6mm*5µ)
Injector : Rheodyne Injector
Operating temperature : Room temperature
Mobile phase : Acetonitrile :phosphate buffer: (40:60)
Flow rate : 1 ml/min
Detection : 231nm
Injection volume : 20µl
Run time : 7 min

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PREPARATION OF SOLUTIONS:

Preparation of Zolpidem Tartrate And Melatonin Standard Stock Solutions:
Accurately weighed quantity of 25 mg zolpidem and 15 mg melatonin was transferred to a 50 mL volumetric flask, dissolved in 15 mL of mobile phase, sonicated for 15 min and the volume was made up to 50 mL, with mobile phase so that the concentration of stock solutions is 0.5 mg/ml of zolpidem tartrate and 0.3 mg/ml for melatonin.

Preparation of Working Standard Solutions:
From the standard stock solutions prepared above the Working standards were prepared by diluting the (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6) mg/ml of the stock solution in six different 10 mL volumetric flasks and adjusted to the mark with the mobile phase to give the following concentrations: 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL and 30 µg/mL. The concentrations obtained for melatonin were: 3 µg/mL, 6 µg/mL, 9 µg/mL, 12 µg/mL, 15 µg/mL and 18 µg/mL.

Preparation Of Buffer:
Accurately weighed quantity of 1.625 gm of potassium dihydrogen phosphate and 0.300 gm of dipotassium hydrogen phosphate were dissolved in 1000 mL of water.

SELECTION OF MOBILE PHASE:
The solution of zolpidem tartrate and melatonin was injected into the hplc system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally acetonitrile and phosphate buffer (40:60v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for zolpidem tartrate and melatonin.

PREPARATION OF MOBILE PHASE:
The mobile phase consisted of acetonitrile and phosphate buffer in the ratio of 40:60(v/v). The pH of the mobile phase was adjusted with ortho phosphoric acid in the double distilled water. Mobile phase was filtered through a 0.45-µm membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1 ml/min).

SELECTION OF ANALYTICAL WAVELENGTH:
From the standard stock solution, further dilutions were prepared using mobile phase and scanned over the range of 200–400 nm and the spectrum was overlain. How ever the detection was carried at 231 nm for zolpidem and melatonin was preferred on the basis of higher response. Hplc run at 231 nm has been found to be better with respect to resolution of the peaks and balanced area acquisition of both drugs. Hence, wavelength of 231 nm was finalized for the data acquisition in HPLC for the simultaneous estimation of both the drugs.

RESOLUTION OF TWO STANDARD DRUGS:
The column was saturated with mobile phase before giving a sample several injection of blank (acetonitrile) was given then another blank injection (mobile phase) was given. Then injections for individual drug were given to get chromatagrams. Combination of standard solution of zolpidem and melatonin was injected. The retention time of both drugs was found to be 3.660 for Zolpidem tartarate: and 2.51 for Melatonin:

ASSAY OF TABLET COMBINATION:
Brand name: Zolosoma FC TAB
Each tablet content: Zolpidem tartrate: 5mg
Melatonin: 3mg
Manufacturer: Pulse manufacturers

Method Validation:

Linearity:
Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph. The calibration curves were plotted over a concentration range of 5-30 mg/ml for zolpidem tartarate and 3-18mg/ml melatonin. Accurately measured standard stock solutions of each zolpidem and melatonin were prepared by diluting the (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6) mL of the stock solution in six different 10 mL volumetric flasks and diluted up to the mark with distill water with the mobile phase to give the following concentrations. The absorbance of solution was then measured at 231 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated. The linearity plots and curves are shown in the figure 3 and 4. Linearity ranges are shown in (table 1)

![Fig.3. Linearity curves of zolpidem and melatonin](image_url)
Precision:
The assay was carried out of two drugs using proposed method in six replicates. The value of relative standard deviation lie well within the limits (0.48% for zolpidem and 0.47% for melatonin), it indicates the sample repeatability of the method enclosed here in (Table 2).

System Suitability:
System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) were determined. The results are shown in (Table 4); it indicates good performance of system.

Accuracy:
Accuracy of the method was determined by applying the proposed method to sample tablet powder containing known amount of each drug to 80%, 100%, and 120% of the label claim. The accuracy was then calculated as the percentage of analyze recovered by the assay. The results of the recovery analysis are enclosed under (Table 3).

Limit Of Detection (Lod): The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula:

$$LOD = \frac{3.3\sigma}{S}$$

Where, $\sigma$ = standard deviation of the response, S = slope of calibration curve.

Limit Of Quantitation (Loq): The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula:

$$LOQ = \frac{10\sigma}{S}$$

Where, $\sigma$ = standard deviation of the response, S = slope of calibration curve.

The values of Theoretical plates, Lod, Loq and Resolution are shown in the (Table4).
Robustness:
The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters. The typical variations are given below: Variation in flow rate by ± 0.1ml/min. Variation in wavelength by ± 2nm.

RESULT AND DISCUSSION OF RP-HPLC METHOD DEVELOPMENT:
The objective of the proposed work was to develop methods for the Determination of zolpidem and melatonin and to validate the methods according to USP and ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of above combination. The present developed HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of zolpidem and melatonin in commercial dosage forms. In RP-HPLC method, Hplc conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to elute title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor, run time, resolution). The instruments used for method development was the hplc system shimadzu with model prominence and the software spinchrome 21CFR, equipped with uv detector source of deuterium lamp,C-18 (250MM*4.6MM*5µ)column and mobile phase comprising of acetonitrile: phosphate buffer(40:60).Different mobile phase was tried and mobile phase used was acetonitrile:phosphate buffer which satisfactorily gives symmetrical and well resolved peak for zolpidem and melatonin. The retention time for zolpidem tartarate and melatonin were 3.660 and 2.51 respectively flow rate kept at 1ml/min and uv detection performed at max 231 nm. The method was validated as per ICH guidelines linearity for detector was observed in 5-30µg/ml for zolpidem and 3-18µg/ml for melatonin respectively percentage recovery of both drug was found in range 99.94-101.5 % indicating accuracy of proposed method the intra-day and inter day coefficient for zolpidem and melatonin were found to be 0.38-0.48%, 0.52-0.47% and 0.04-0.43%, 0.00-0.78% the percentage RSD for both the tablet analysis and recovery studies is less than 2% indicating high degree of precision . The result of robustness study also indicate that the method is robust and is unaffected by small variation in chromatographic condition. It was observed that excipient present in formulation did not interfere with peaks of zolpidem and melatonin. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robust, accuracy, linearity and precision without any prior separation step. HPLC method generates large amounts of quality data which serve as highly powerful and convenient analytical tool.

CONCLUSION:
From all results it can be conclude that the developed RP-HPLC method is simple, sensitive, accurate, precise, and selective. Percentage recovery shows that the method is free from interference of excipients used in the formulation.

ACKNOWLEDGEMENT:
The authors are thankful to the Management of Brown’s College of Pharmacy for granting permission for the publication of this work.

REFERENCES:
10. Sai krishna.namburi , s.v.u.m.prasad , d.venkat rao , k.manga , k.tarun krishna. Rp-hplc method development and validation for theSimultaneous estimation of melatonin and zolpidem Tartrate in tablet dosage form, journal of advances in drug research,vol II, Issue 1, Jan 2012

Source of support: Nil, Conflict of interest: None Declared