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# DEVELOPMENT AND VALIDATION OF NOVEL ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF MELATONIN AND ZOLPIDEM TARTRATE IN PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC TECHNIQUE

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**ABSTRACT**

A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of melatonin and zolpidem tartrate in tablets. The chromatographic separation was carried out on $C_{18}$ ODS Hypersil -(250X4.6mm, 5µ) with a mixture of mixed phosphate buffer : acetonitrile (55:45%v/v) as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 265 nm. The retention times were 2.517 and 3.630 min for melatonin and zolpidem tartrate respectively. Calibration plots were linear ($r^2$=0.999) over the concentration range of 6-42 µg/mL for melatonin and 10-70 µg/mL for zolpidem tartrate. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of melatonin and zolpidem tartrate in bulk and tablet dosage form.

**Keywords:** Melatonin, Zolpidem tartrate, RP-HPLC, Tablets.

## INTRODUCTION

Zolpidem tartrate (Fig. 1) (ZOL) is a prescription medication used for the short term treatment of insomnia, as well as some brain disorders, short - acting non benzodiazepine hypnotic of the imidazopyridine class that potentiates gamma – amino butyric acid (GABA), an inhibitory neurotransmitter, by binding to GABA-A receptors at the same location as benzodiazepines. It works quickly (usually within 15minutes) and has a short half-life (2–3 hours). Melatonin (MELA) is a biogenic amine that is found in animals, plants and microbes (Fig. 2). Chemically it is N-[2-(5-methoxy-1H-indol-3-yl) ethyl] ethanamide. Melatonin regulates the sleep-wake cycle by chemically causing drowsiness and lowering the body temperature. Melatonin is also implicated in the regulation of mood, learning and memory, immune activity, dreaming, fertility and reproduction. Melatonin is also an effective antioxidant. Most of the actions of melatonin are mediated through the binding and activation of melatonin receptors. Reduced melatonin production has also been proposed as a likely factor, in the significantly higher cancer rates in night workers[1-4].

Literature survey reveals that few spectrophotometric[5-6]and chromatographic methods[7-17]were reported for estimation of MELA and ZOL in single and combination with other drugs. Therefore, an attempt has been made to develop an accurate, rapid and reproducible reverse phase HPLC method for simultaneous determination of MELA and ZOL in tablet dosage form and validate it, in accordance with International Conference on Harmonization (ICH)[18-19] guidelines.

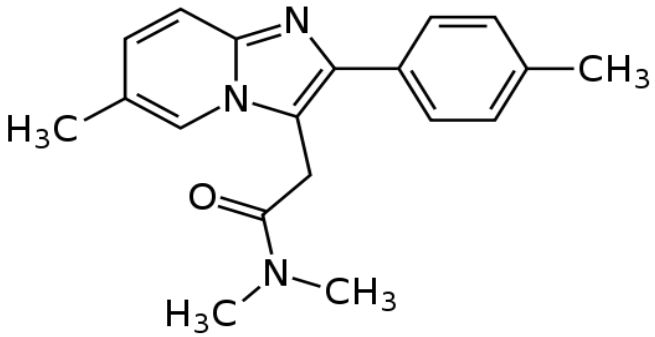


**Fig. 1: Molecular structure of zolpidem tartarate**

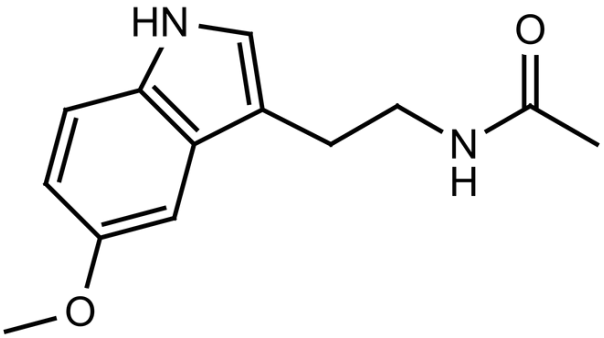


**Fig. 2: Molecular structure of melatonin**

## MATERIAL AND METHOD

### Chemicals and reagents

The reference samples of MELA (API) and ZOL (API) were obtained from Pulse Pharmaceuticals, Hyderabad. The branded formulations ZOLSOMA FC tablets were procured from the local market. Tablets claimed to contain 3mg of MELA and 5mg of ZOL have been utilized in the present work. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

### Chromatographic conditions

Separation was performed on an isocratic Shimadzu 1100 series HPLC instrument equipped with a LC-20 AT Vp Series fitted with binary pump and variable wavelength UV-Visible detector, SPD-20A. A 20µL Hamilton syringe was used for injecting the samples. Data was analysed by using spinchrome software. Elico SL 159 UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials. The separation was achieved on a $C_{18}$ ODS Hypersil - (250X4.6mm, 5µ) analytical column. The mobile phase consisted of mixed phosphate buffer: acetonitrile (55:45%v/v). The flow rate was 1.0 mL/min and UV detection was performed at 265 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent

mobile phase was filtered through a 0.45 µ membrane filter (Millipore, Ireland). The injection volume was 20 µL and all the experiments were performed at ambient temperature.

**Preparation of standard solution**

Accurately weighed quantity of 15 mg of Melatonin and 25 mg of Zolpidem tartrate was transferred to a 50 ml volumetric flask, dissolved in 30 ml of mobile phase, sonicated for 15 min and the volume was make up to 50ml with mobile phase. The stock solution (Melatonin: 300µg/ml, ZolpidemTartrate: 500µg/ml) was used in the study after appropriate dilution.

**Preparation of sample preparation**

Accurately weighed 10 tablets, average weight is taken and powdered. Accurately weighed powder equivalent to 25 mg zolpidem tartarate and 15 mg of melatonin was accurately weighed and taken in a 50ml volumetric flask and 30 ml of mobile phase was added. The mixture was subjected to sonication for 15min with intermediate shaking for complete extraction of drugs. Filtered through a whatmann filter paper and cooled to room temperature and solution was made up to mark with mobile phase.

**Method validation**

The developed method was validated according to ICH guidelines. The system suitability was evaluated by five replicate analysis of MELA and ZOL mixture at concentrations of 30 µg/mL and 50 µg/mL. The acceptance criteria are theoretical plates numbers (N) at least 3000 per each peak and tailing factors not more than 2.0 for MELA and ZOL.

**Linearity**

Standard calibration curves were plotted against the concentration ranging from 6-42 µg/mL for MELA and 10-70 µg/mL for ZOL. Different linearity levels was prepared and injected into the HPLC system keeping the injection volume constant.

**Recovery**

To study the reliability and suitability of developed method, recovery experiments were carried out at three levels 80%, 100% and 120%. Known concentration of commercial tablet was spiked with known amount of MELA and ZOL. At each level, three determinations were performed with expected results. The %RSD of individual measurements was also determined.

**Precision**

Precision of assay was determined by System and Method Precision. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD.

**Specificity**

All chromatograms were examined to determine whether compound of interest co eluted with each other or with any additional excipient peaks. Marketed formulation was analysed to determine the specificity of the optimized method in presence of common tablet excipients.

**Limit of detection and limit of quantification**

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

**Robustness**

To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included are variation of flow rate and Detection Wavelength.

**RESULTS AND DISCUSSION**

During the optimization of HPLC method, two columns symmetry C-18 and C-8 analytical column (4.6×250 mm; 5 µm) and (4.6×150 mm; 5 µm), two organic solvents (acetonitrile and methanol), one buffer (phosphate) were tested. Initially Water:Actonitrile and Methanol, acetonitrile:Phosphate buffer, were tried in different ratios. Finally mobile phase consisting of mixture of acetonitrile:Phosphate buffer in ratio 45:55 (v/v) was selected as mobile phase to achieve clear separation and sensitivity. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal to noise ratio with reasonable separation time using a $C_{18}$ analytical column (4.6×250 mm; 5 µm), the retention times for MELA & ZOL were observed to be 2.517 and 3.630 min respectively. Total run time was less than 8 min. The chromatogram at 265 nm showed a complete resolution for all peaks (Fig. 3). Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability tests. All critical parameters tested meet the acceptance criteria on all days. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks.

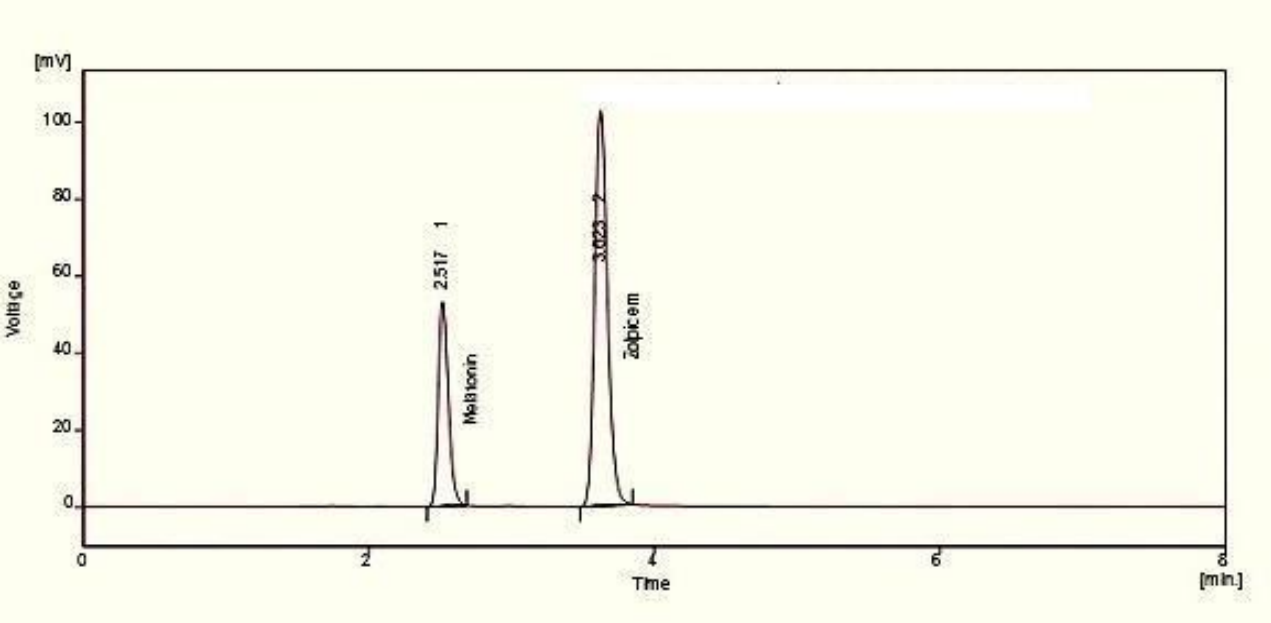


**Fig. 3: Typical chromatogram of standard for MELA and ZOL**

Linearity was obtained for MELA & ZOL in the range of 6-42 µg/mL and 10-70 µg/mL. The correlation coefficient ($r^2$) was found to be greater than 0.999 in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for MELA & ZOL in tablet dosage form. Results obtained from recovery studies presented in Table 2 indicate that this assay procedure can be used for routine quality control analysis of binary mixture

in tablets. Precision of the analytical method was found to be reliable based on %RSD (<2%) corresponding to peak areas and retention times. As can be seen in Table 3 the %RSD values were less than 2 for System & Method precision. Hence, the method was found to be precise for these two drugs.

**Table 1: System suitability parameters of proposed method**

| S. No. | Parameters | Melatonin | Zolpidem |
|---|---|---|---|
| 1 | Linearity | 6-42µg/ml | 10-70 µg/ml |
| 2 | Theoretical plates | 5893 | 7473 |
| 3 | Tailing Factor | 1.464 | 1.352 |
| 4 | Resolution | -- | 7.168 |
| 5 | LOD | 0.031 µg/mL | 0.212 µg/mL |
| 6 | LOQ | 0.108 µg/mL | 0.641µg/mL |

**Table 2: Accuracy data for proposed method**

| Spiked level of drug (%) | Amount of drug added (µg/ml) | | %Mean recovery (n=6) | | %RSD | |
|---|---|---|---|---|---|---|
| | MELA | ZOL | MELA | ZOL | MELA | ZOL |
| 80 | 12 | 20 | 99.5 | 99.5 | 0.19 | 0.27 |
| 100 | 15 | 25 | 99.8 | 99.6 | 0.14 | 0.39 |
| 120 | 18 | 30 | 99.5 | 99.6 | 0.09 | 0.16 |

**Table 3: Precesion data of proposed method**

| Sample No. | SYSTEM PRECISSION | | METHOD PRECISSION | |
|---|---|---|---|---|
| | MELA | ZOL | MELA (%ASSAY) | ZOL (%ASSAY) |
| 1. | 351.700 | 751.396 | 99.30 | 99.98 |
| 2. | 349.651 | 750.669 | 99.42 | 99.18 |
| 3. | 350.232 | 752.235 | 99.33 | 99.89 |
| 4. | 352.312 | 750.152 | 99.61 | 100.21 |
| 5. | 351.450 | 751.682 | 99.32 | 99.96 |
| Mean | 351.069 | 751.326 | 99.48 | 99.81 |
| S.D. | 1.095 | 0878 | -- | -- |
| %RSD | 0.32 | 0.11 | 0.11 | 0.34 |

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and percentage purity calculated was found to be within limits. LOD and LOQ were found to be 0.031 µg/mL and 0.108 µg/mL for MELA, 0.212 µg/mL and 0.641 µg/mL for ZOL. In all deliberately varied conditions, the %RSD for replicate injections of MELA & ZOL were found to be within the acceptable limit. The tailing factors for two peaks were found to be less than 1.5 and the results are shown in Table 4. The validate method was used in analysis of marketed tablet dosage form. The results for the drugs assay showed good agreement with label claims and the results are shown in Table 5.

**Table 4: Robustness for flow rate variation of MELA and ZOL**

| FLOW RATE | Rt of MELA | Rt of ZOL |
|---|---|---|
| 0.9ml/min | 2.720 | 3.917 |
| 1.1ml/min | 2.337 | 3.367 |

**Table 5: Analysis of marketed formulation by proposed method**

| Brand name | DRUG | Label claim (mg) | Amount Found (mg) | % Label claim |
|---|---|---|---|---|
| ZOLSOMA FC | MELA | 3 | 2.99 | 99.64 |
| | ZOL | 5 | 4.99 | 99.73 |

## CONCLUSION

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of MELA & ZOL in tablet dosage form. The developed method provides good resolution between MELA & ZOL. It was successfully validated in terms of system suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

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