

RP-HPLC Method Development and Validation for Simultaneous Estimation of Alprazolam and Melatonin in Pharmaceutical Dosage Form

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ABSTRACT

Melatonin a hormone is a major product of the pineal gland. Alprazolam is an anti-anxiety drug that belongs to the benzodiazepines category. The quantity of alprazolam and melatonin had been calculated by the use of a regression equation and the responses were measured at 277 nanometers wavelength using the above-mentioned conditions RP-HPLC simultaneous estimation of Alprazolam and Melatonin has developed a method and determined for validation. The method was estimated as specific and no interference was seen from impurities and excipients. The present study method developed as simple, precise, and accurate for determination and can be further used for the analysis of Alprazolam and Melatonin for dosage formulation.

Keywords: Method validation, RP-HPLC, Alprazolam, Melatonin, Dosage form
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INTRODUCTION

Melatonin (N-acetyl-3-(2-aminoethyl)-5-methoxyindole), an endogenous hormone, is a predominant product of the pineal gland. Melatonin shows a pharmacological activity in the management of Alzheimer's diseases, Parkinson's disease, depressive disorder, glaucoma, breast and prostate cancer, hepatoma, and melanoma.^[1,2]

Alprazolam is an anti-anxiety drug that belongs to the benzodiazepines class. It is chemically 8-Chloro-methyl-6-phenyl-4H-[1,2,4] triazo[4,3-a][1,4]benzodiazepine having the molecular formula C₁₇H₁₃C₁N₄ and molecular weight 308.769 g/mol.^[3,4] It affects that the neurotransmitters present in the brain which are unbalanced and are not stable during anxiety.^[5,6] It works by enhancing the effects of certain natural chemicals (GABA) in the body.^[7-9] Alprazolam is clinically useful in the treatment of depression disease as a new antidepressant drug used in the treatment of anxiety and depression with tension.^[10] A literature survey revealed the development of various spectrophotometric, HPTLC, and HPLC method for the estimation of Melatonin and Alprazolam in various dosage forms individually.^[11-13] It was found that only a few methods are available for the simultaneous estimation of melatonin and alprazolam drugs. Here, these two combinations were selected for HPLC method development and validation. The structure of Alprazolam and Melatonin is shown in Figure 1.

MATERIALS AND METHODS

Instrument

The chromatography method was developed by an HPLC series equipped with a UV-visible detector that was used. The apparatus and instruments are calibrated according to the Bureau of Standards.

Chemical

The medicines Alprazolam and Melatonin (0.25 mg and 3 mg) were taken as gift samples from the Cipla

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Pharmaceutical Limited Jammu. The tablets of Alprazolam and Melatonin (0.25 mg and 3 mg) were purchased from the local market.

RP-HPLC Estimation

Chromatographic condition and mobile phase

The mobile phase composition is acetonitrile, methanol, and water in the different ratios selected by the hit and trial method. The mobile phase is filled in the reservoirs. The gradient pumping is used the pump the mobile phase by changing the ratio according to the time.

Wavelength detection

The wavelength for maximum absorption is observed by scanning the medicament sample Alprazolam and Melatonin in the range of 200–800 nanometers in a double beam ultra-violet double beam spectrophotometer (Shimadzu 1800 model). Detection was carried out at different wavelengths but the best response was achieved at 277 nanometers.

Preparation of Alprazolam and Melatonin standard stock solutions

The 10 mg of Alprazolam and 120 mg of Melatonin were taken on butter paper and transferred to the digital weighing balance pan and weighed accurately. The weighed amount of Alprazolam (10 mg) and Melatonin (120 mg) was transferred to the 100-ml capacity volumetric flask. The dilution is done with distilled water to obtain a concentration of 100 micrograms/milliliter of Alprazolam and 1200 micrograms/milliliter of Melatonin taking drug solution from the stock solution.

Preparation of sample solution

Powdered the correctly weighed 20 tablets and quantity was taken equivalent to 0.25 mg of Alprazolam and 3.0 mg of Melatonin. The content was transferred into the 10-ml volumetric flask and volume was done by the mobile phase. The solution was sonicated for 20 min and after that solution was filtered. 0.8 ml from this solution was withdrawn and diluted with mobile phase into 10 ml volumetric flask.

Method Validation

Calibration curve (linearity)

Standards working solution for Alprazolam and Melatonin (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml) was taken. For this concentration range was selected 1–10 micrograms/milliliter for Alprazolam and 12–120 micrograms/milliliter for Melatonin.

Accuracy (% recovery)

The recovery studies had been produced by adding the bulk drug in the ratio of 50%, 100%, and 150%. The correctness of the method was evaluated by producing triplicates of each and statistical data were produced using the analysis of variance method with software like SPSS on the computer. Three concordance readings were found to be near true values of the results and validatory for the method.

Method precision (% repeatability)

Standard solutions of Alprazolam (4 microgram/milliliter) and Melatonin (48 microgram/milliliter) were selected for the measurement of instrument precision. Precision is related to the repeatability of the developed method.

Intermediate precision (reproducibility)

The concentrations for standard solution of Alprazolam (2.0, 4.0, and 6.0 microgram/milliliter) while for Melatonin (24.0, 48.0, and 72 microgram/milliliter) were selected for the measurement of intraday and interday precision.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD is the minimum amount that could be detected from the sample and the LOQ is the amount that could be quantified from the sample. %LOD and LOQ had been found by the equation.

Analysis of alprazolam and melatonin in combined dosage

The quantity of Alprazolam and Melatonin had been calculated by the use of a regression equation and the responses were measured

at 277 nanometers wavelength using the above-mentioned conditions.

RESULTS

In this chromatogram, Alprazolam and Melatonin were separated by mobile stage said 60 parts ACN: 30 parts Methanol: 10 parts water. From the chromatogram, it had been concluded that the evaluation is very faster and the run time was also 5 min as shown in Figure 2.

Linearity

Calibration curves of alprazolam (1-10 µg/milliliter) and Melatonin (12-120 µg/milliliter) with different concentration was observed at 277 nanometer respectively.

Accuracy

The recoveries obtained were $99.99 \pm 0.41\%$ and $100.62 \pm 0.54\%$ for Alprazolam and Melatonin, respectively.

Intermediate Precision (Reproducibility)

According to procedure conducted, Interday precision for alprazolam and melatonin was found 0.196-0.453% and 1.61-0.867% while for Intraday precision was found (0.047-0.285% and 0.010-0.155%) respectively.

LOD and LOQ

Alprazolam and Melatonin LOD had been observed $0.016 \mu\text{g ml}^{-1}$ and $0.52 \mu\text{g ml}$, in turn, while LOQ had been observed at 0.048 microgram/milliliter and 1.57 microgram/milliliter for the same.

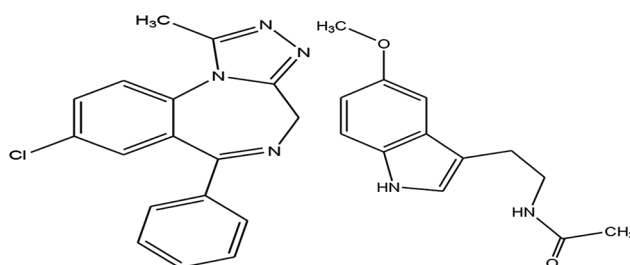


Figure 1: (a) Alprazolam structure (b) Melatonin structure^[2,3]

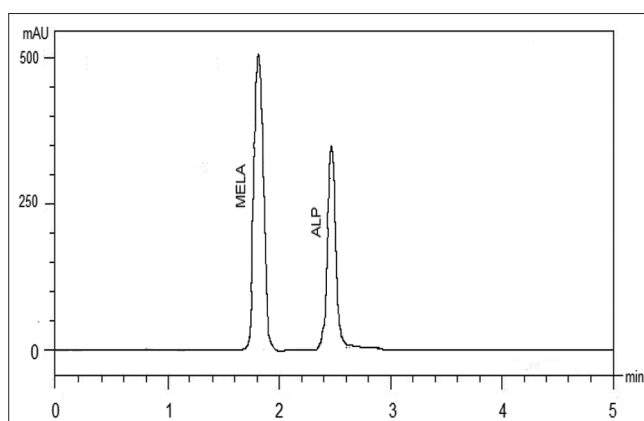


Figure 2: RP-HPLC chromatogram of alprazolam and melatonin

Precision (% Repeatability)

Alprazolam and Melatonin were found to be 0.36% and 0.24%, respectively, which is <2%. Hence, the method is precise.

Assay of the Pharmaceutical Formulation

Alprazolam and Melatonin were related to the consistently labeled extents.

CONCLUSION

RP-HPLC simultaneous estimation of Alprazolam and Melatonin has developed a method and determined for validation. The method was estimated as specific and no interference was seen from impurities and excipients. The method developed was simple, precise, and accurate for determination and can be further used for the analysis of Alprazolam and Melatonin for dosage formulation.

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