

Enantioseparation of (*RS*)-Zopiclone using amino acid through thin layer chromaography

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Abstract

Resolution of enantiomers of (*RS*)-Zopiclone was achieved by using direct thin layer chromatography on silica gel plates impregnated with optically pure (*L*-Tryptophan) as chiral selector and as chiral mobile phase additive. The enantiomers were resolved by using the solvent system (acetonitrile, methanol and water) with different ratio. Spots were detected by using chamber.

Keywords: Enantioseparation, Thin layer chromatography, Zopiclone

1. Introduction

Almost a large fraction of active pharmaceutical ingredients are enantiomers and the desired biological activity is provided by only one of the two enantiomers. The undesirable enantiomer results into side effects caused due to different therapeutic and pharmacological activities of one of the enantiomers. With regard to concern at maintaining and enhancing the global health, among the scientific community the US Food and drug administration along with other regulatory bodies has created a general awareness. Consequently the racemic drug dealing has led the regulatory bodies in many countries involved in the registration process of all new active ingredients to pay a potential focus on the registration of single enantiomer of a drug along with the stereo selectivity and stereo specificity of drugs. Stereoselectivity has been encouraged and also well documented in nearly all the pharmacokinetic processes such as absorption, distribution, excretion, etc[1-2]. The living systems being chirally selective cause the two enantiomers of a racemic drug to have different chemical reactivity, potency, efficacy, different pharmacokinetic and pharmacodynamic interaction and also different toxicities. They interact differently towards one same receptor and enzyme; hence resolution is an important process for mankind [3,4]. Since chiral environment is undoubtedly a primary requirement for chiral separation so the chiral environment for the chromatographic resolution can be in a way provided through *L*-amino acids. *L*-Amino acids being enantiomerically pure can be used as chiral selectors, mobile phase additives and also as other chiral auxiliaries to prepare the chiral derivatizing reagents[5-7]. The chosen drug, Zopiclone(Figure 1) 6-(5-chloro-2-pyridyl)-7-(4-methylpiperazinyl) carbonyloxy-6,7-dihydro [5~pyrro~o [3,4-blpyrazin-5-on], which is a non-benzodiazepine hypnotic drug belonging cyclopyrrolone class is commercialized as a racemate[8,9]. It is an anti-depressant. Zopiclone is primarily used for the treatment of insomnia and both the enantiomers follow a different rate of metabolism. (+)-Zopiclone or Eszopiclone has 50-60 times greater receptor binding potency towards the benzodiazepine than (-)-zopiclone [10]. In many countries like Europe and Japan zopiclone is commercialized as a racemate. FDA has approved the enantiomer (+)-zopiclone having higher pharmacological activity compared to the other enantiomer [11]. There have been a few reports on the enantioresolution of Zopiclone using Hydroxy-Propyl-BetaCyclodextrin as Chiral Selector[12], Chiral α -Acid Glycoprotein Column[13], β -cyclodextrin bonded phase [14]. For the enantioseparation of (*RS*)- Zopiclone, this is the first report following this approach

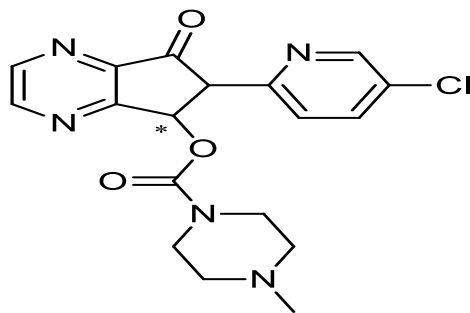


Figure 1: Structure of (RS)- Zopiclone

2. Experimental

2.1. Chemicals and instrumentation:-

UV visible spectrometry was performed by spectrophotometer SHIMADZU-UV-1800. IR analysis was performed using FTIR by SHIMDZU. Pharmaceutical drug marketed as zopiclone by ALKEM Laboratories Ltd. (Mumbai, India) was purchased from a local drug store containing 10mg active pharmaceutical ingredient zopiclone. Silica gel was purchased from LOBA CHEMIE pvt ltd. (Mumbai, India). Amino acid (L-Tryptophan) marketed by Lobachemie Pvt Ltd. (Mumbai, India) and other reagents marketed by Qualikems Fine chem. Pvt Ltd. And ALPHA CHEMIKA (Mumbai India) were obtained from nearby drug store.

2.2. Isolation and purification of (RS)- Zopiclone

30 tablets of racemic zopiclone were taken for purification and was purified by grinding it to fine powder using pistil. The powder so obtained was dissolved into methanol (maximum soluble) and the solution formed in the beaker was stirred properly to get the maximum drug dissolved. Then the solution was sonicated for nearly 20 minutes on the sonicator about 5 to 6 times. The filtration was done using 190µm thick Whatman filter paper having pore size 8µm. The filtrate obtained after the completion of the sonication process was evaporated in air and crystals of pure sample were achieved at room temperature. With the help of IR and UV the sample was analyzed and the peaks of the drug were obtained confirming us the purification of the sample. Also, melting point was taken to check out the purity and was found out to be 129 °C.

2.3. Thin Layer Chromatography

Separation was carried out through direct method using amino acid L-tryptophan as chiral reagents through two different approaches.

Approach A:- Amino acid was impregnated into the TLC plates and resolution of racemic zopiclone was carried out using different achiral solvent systems. The TLC plates were dried and activated for nearly 8 to 10 hours at an appropriate temperature of 50 to 60 °C. Also, to get the desired results, different binary and ternary solvents of methanol, water and acetonitrile were used in different ratios.

Approach B:- Chiral mobile phase was prepared and separation was performed by putting an appropriate amount of chiral selector into the ternary solvent system. Spots of chiral drug were put on the plain TLC plates and this way employed for resolution respectively.

3. Results and Discussion

In approach A, TLC plates were impregnated with L-Tryptophan. Slurry of silica gel was prepared by taking 50ml of distilled water and a 10⁻²M pure amino acid solution was mixed in it. Also, 25g of silica gel were dissolved in the chiral solution prepared and the slurry formed was used to prepare the TLC plates. The TLC plates prepared were dried at room temperature and activated at nearly 60 °C in an oven for 8 hours. After heating the TLC plates, they were cooled to room temperature and used for separation. In approach B, the chiral mobile phase was prepared initially by preparing a 10⁻²M amino acid solution in 95:5 (water : methanol) and added in appropriate ratio to the binary or ternary solvent system.

Chromatograms were developed in iodine chamber for both approach A and B. Different binary and ternary solvent systems using acetonitrile, methanol and water were used. Resolution was not achieved using the binary solvent systems of acetonitrile and methanol. Separation was achieved using a ternary solvent system of acetonitrile, methanol and water. The different ratios tried for resolution were 4:3:3, 5:1:1, 5:4:5 etc. The successful ratios of resolution are given in the Table 1. Spots were located in an iodine chamber. Chromatograms achieved showing resolutions through this method are given below in Figure 2. It was observed that via approach A resolution was found to be 4.21 and via approach B it was 3.89. Approach A was found to good as compare to Approach B. Between 16 and 32 °C analyze with effective solvent systems, were done. For this purpose, those chromatographic chambers were set inside an incubator to accomplish the particular temperature. Those best determination to (*RS*)- Zopiclone for constantly on L- tryptophan for both approach acquired during 28 °C. Expand about temperature should 32°C brought about tailing from claiming spots Also adiminish clinched alongside temperature to 16°C demonstrated no determination or eight molded structures.

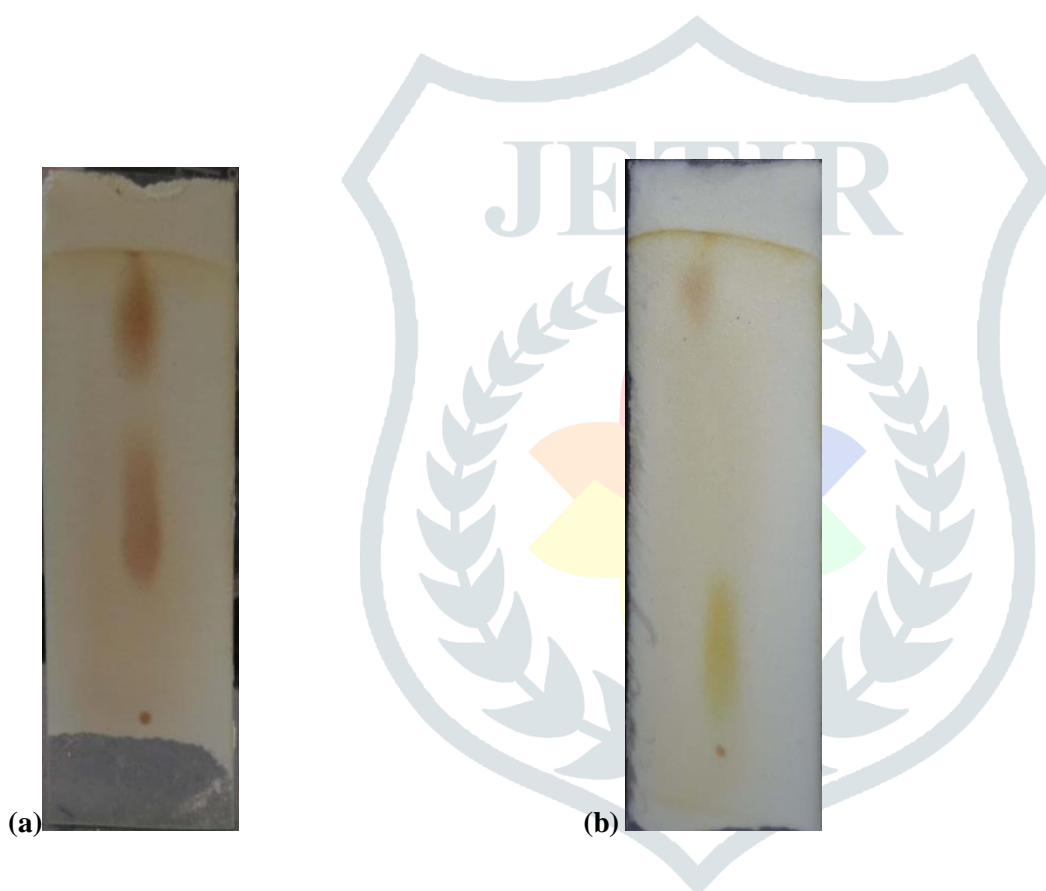


Figure 2: Pictures of chromatograms showing resolution using (a) approach A, (b) approach B successful solvent system:-Acetonitrile:Methnaol:Water

Table 1: For enantioseparation of (RS)-Zopiclone using L- Tryptophan the R_s and hR_f ($R_f \times 100$) values are shown below:

Approach	Solvent system	hR_f		R_s
		R_{f1}	R_{f2}	
A	Acetonitrile-methanol-water(4.3:3 v/v)	32	64	4.21
B	Acetonitrile-methanol-water(4.4:2 v/v)	14	93	3.89

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