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Enantiomeric separation of *S*-zopiclone and its *R*-enantiomer in bulk drug samples by validated chiral RP-HPLC

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A simple and selective isocratic chiral RP-HPLC method was developed for the enantiomeric purity determination of *S*-zopiclone and the quantitative determination of *R*-zopiclone in bulk drug samples. Enantiomeric separation was achieved using Chiralcel OD-RH 150 × 4.6 mm, 5 μm particle size column at 25 °C using a mobile phase of 10 mM ammonium acetate and acetonitrile in ratio of 60:40 (*v/v*) as mobile phase at a flow rate of 1.0 mL.min^{-1} and UV detection at 306 nm. The method resolves the *R*-zopiclone and *S*-zopiclone with resolution (*Rs*) greater than 1.6. The limit of detection (LOD) and limit of quantitation (LOQ) of the *R*-enantiomer were 0.12 μg. mL^{-1} and 0.40 μg. mL^{-1} respectively, for 10 μL injection volume. The percentage RSD of the peak area of six replicate injections of *R*-zopiclone at LOQ concentration was 4.6. The percentage recoveries of *R*-enantiomer from *S*-zopiclone were ranged from 97.3 to 99.8. Developed method was found to be selective in presence potential impurities. The developed chiral RP-HPLC method was validated with respect to precision, linearity, accuracy, robustness and ruggedness. The test solution and mobile phase was found to be stable up to 24 h after preparation.

1. Introduction

Analytical methods to determine the enantiomeric purity of new investigational drugs are often attained through a series of generic or screening methodologies. Although many analytical techniques can be employed to achieve this, the most widely used is liquid chromatography (LC) employing a chiral stationary phase (CSP).

S-Zopiclone (5*S*)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate is a short-acting nonbenzodiazepine hypnotic agent developed for the treatment of insomnia, has been available in Europe since 1992 and in the US since 2005.

Fernandez et al. (1991) reported the separation and determination of zopiclone enantiomers in human plasma using a sequential achiral-chiral liquid chromatographic method. Zopiclone was separated from the biological matrix and quantified on an achiral silica column. Foster et al. (1994), described a normal-phase liquid chromatographic method for determination of zopiclone enantiomers in human plasma samples. Enantioseparation was achieved on Chiralcel OD-H column using a mobile phase consisting of ethanol-hexane (60:40 *v/v*) delivered at flow rate of 0.6 mL. min^{-1} with fluorescence detection. Gebauer and Alderman (2002), reported quantitative approaches for determination of zopiclone enantiomers in human plasma samples based on chromatographic conditions. Mannaert and Daenens described the zopiclone enantiomers separation on a Chiralpak AS column under semi-preparative LC conditions. Since, the first three papers are mainly described the separation and determination of zopiclone enantiomers in human plasma samples only. Mannaert and Daenens described the semi-preparative liquid chromatographic conditions but no details were discussed on quantitative determination of enantiomers at lower concentration in bulk drug samples.

To our present knowledge there are no HPLC methods available for separation and quantitative determination of *S*-zopiclone and its *R*-enantiomer in bulk drug samples. The present research work developed a simple and accurate chiral HPLC method for the separation of *S*-zopiclone and its undesired *R*-enantiomer in bulk drug samples. This paper also deals with the method development and validation.

2. Investigations, results and discussion

S-Zopiclone and its *R*-enantiomer samples were individually prepared and used during method development. Different normal phase and reversed phase chiral stationary phases were employed namely Chiralpak AD-H, Chiralcel OD-H, Chiralpak IC, Chiralpak AD-RH, Cyclobond I 2000 and Chiralcel OD-RH. Separation between *S*-zopiclone and its *R*-enantiomer was achieved by using the chiralcel OD-RH column with 10 mM ammonium acetate and acetonitrile in ratio of 60:40 (*v/v*) as a mobile phase. Different trials were made during the method development and the details were summarized in Table 1.

Chromatographic separations were achieved on Chiralcel OD-RH (150 × 4.6 mm, 5 μm particle size, Make: Daicel Chemical Industries Ltd., Japan) chiral column. The chiral stationary phase (CSP) present in Chiralcel OD-RH columns is tris (3, 5-dimethylphenylcarbamate) cellulose derivative coated on silica gel. The mobile phase system contained the mixture of

Table 1: Results of various trials

Trial No.	HPLC conditions	Remarks
1	Column: Chiralpak AD-H 250 × 4.6 mm, 5 µm Mobile phase: methanol: ethanol and DEA (25:75:0.1, *v/v*) Flow rate: 1.0 mL. min^{-1} Column temperature: 25 °C	R_S between the pair of enantiomers: 2.6 Longer retention (t_R): 33.4 min
2	Column: Chiralpak AD-H 250 × 4.6 mm, 5 µm Mobile phase: heptane: IPA and DEA (25:75:0.1, *v/v*) Flow rate: 0.8 mL. min $^{-1}$ Column temperature: 25 °C	R_S between the pair of enantiomers: 2.4 Longer retention (t_R): 24.9 min
3	Column: Chiralcel OD-H 250 × 4.6 mm, 5 µm Mobile phase: methanol: ethanol and DEA (60:40:0.1, *v/v*) Flow rate: 1.0 mL. min $^{-1}$ Column temperature: 25 °C	R_S between the pair of enantiomers: 2.3 Observed broad peak shape
4	Column: Chiralpak IC 250 × 4.6 mm, 5 µm Mobile phase: heptane: ethanol (25:75, *v/v*) Flow rate: 1.0 mL. min $^{-1}$ Column temperature: 25 °C	R_S between the pair of enantiomers: > 5.0 Longer retention (t_R): 50.7 min
5	Column: Chiralpak IC 250 × 4.6 mm, 5 µm Mobile phase: methanol: ethanol (50:50, *v/v*) Flow rate: 1.0 mL. min $^{-1}$ Column temperature: 25 °C	R_S between the pair of enantiomers: > 3.0 Longer retention (t_R): 30.5 min
6	Column: Chiralpak AD-RH 150 × 4.6 mm, 5 µm Mobile phase: 10 mM ammonium acetate: acetonitrile (10:90, *v/v*) Flow rate: 1.0 mL. min $^{-1}$ Column temperature: 25 °C	R_S between the pair of enantiomers: 2.3 Observed broad peak shape.
7	Column: Cyclobond I 2000 250 × 4.6 mm, 5 µm Mobile phase: 10 mM ammonium acetate: acetonitrile (80:20, *v/v*) Flow rate: 1.0 mL. min $^{-1}$ Column temperature: 25 °C	*R* and *S*-enantimers are co-eluted
8	Column: Chiralcel OD-RH 250 × 4.6 mm, 5 µm Mobile phase: 10 mM ammonium acetate: acetonitrile (60:40, *v/v*) Flow rate: 1.0 mL. min^{-1} Column temperature: 25 °C	R_S between the pair enantiomers :1.8, Observed good selectivity and peak shape.

10 mM ammonium acetate and acetonitrile (60:40 *v/v*). The flow rate was kept at 1.0 mL. min^{-1}. The sample concentration was 0.5 mg. mL^{-1} in acetonitrile (diluent). The columns temperature was maintained at 25 °C and the elution was monitored at 306 nm. The injection volume was 10 µL. The total analysis time for each run was 20 min.

Good separations were achieved within a short runtime on Chiralcel OD-RH column (*USP* resolution was found to be > 1.6). The typical retention times of *S*-zopiclone, *R*-zopiclone, Imp-1 and Imp-2 were 6.2, 5.4, 2.7 and 12.8 min respectively (Fig.). The system suitability (Swartz and Krull 1998) results are listed in Table 2. In the optimized method, the results were indicative of suitability for the purpose intended.

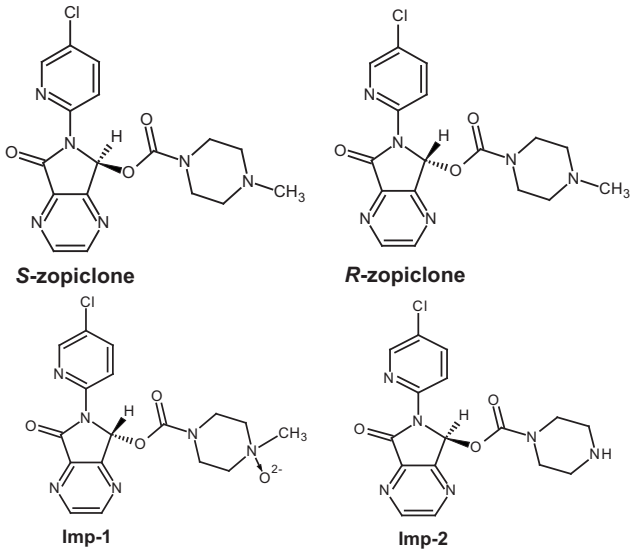
3. Experimental

3.1. Chemicals and reagents

Samples of *R*-zopiclone, *S*-zopiclone, (5RS)-6-(5-chloropyridin- 2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-b]pyrazin-5-yl4-methylpiperazine-1-carboxylate 4-oxide (Imp-1) and (5*S*)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*] pyrazin-5-yl piperazine-1-carboxylate (Imp-2) samples were obtained from Process Research Department, Bulk Actives Unit-3, a business unit of Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile, IPA, CH3OH and ammonium acetate were purchased from Ranbaxy fine chemicals, New Delhi, India. Ethanol, heptane and di ethyl amine (DEA) were purchased from Qualigens fine chemicals, Mumbai, India.

Table 2: System suitability test results

Name	Retention time (t_R) in min	Resolution (R_s) by Tangent method (*USP*)	*USP* Tailing factor (*T*)
R-Zopiclone	5.4	-	1.4
S-Zopiclone	6.2	1.8	1.5



3.2. Instrumentation

The HPLC system used for method development, peak homogeneity verification and method validation was an Agilent 1100 series (Agilent Technologies Inc., Palo Alto, CA, USA) with DAD detector and Waters alliance system (Milford, USA) with a 2695 separation module. The output

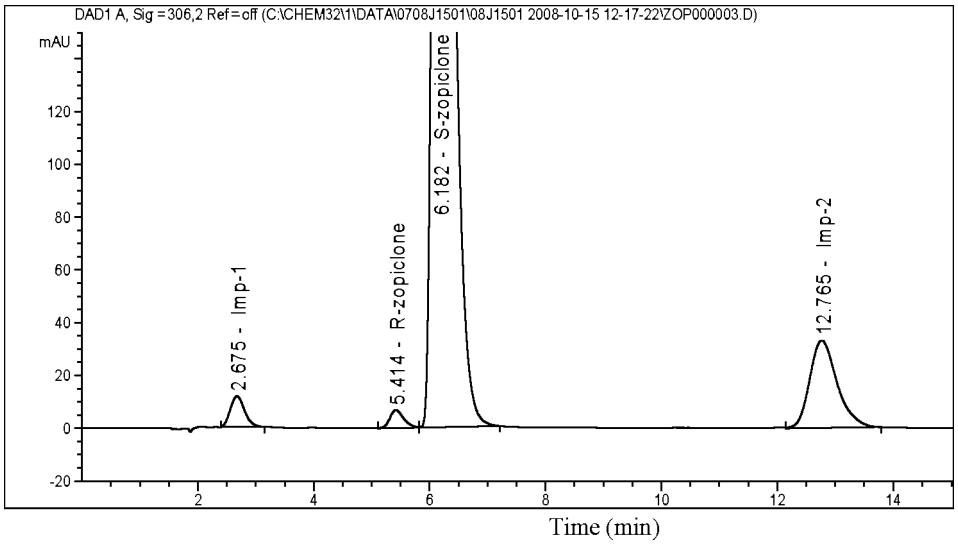


Fig.: Typical selectivity HPLC chromatogram

signal was monitored and processed using Chemstation software (Agilent) and Empower software (Waters) on Pentium computer (Digital Equipment Co.). Photo stability studies were carried out in a Sanyo photo stability chamber (Leicestershire, UK) and thermal stability studies were performed in a hot air oven (Thermo instruments, India).

3.3. Sample preparation

An amount of 25 mg each *R*-zopiclone (99.8% pure) and *S*-zopiclone (99.5% pure) was dissolved in 50 mL of acetonitrile (diluent). The resultant solution had 0.5 mg mL^{-1} of individual concentration. The target analyte concentration of *S*-zopiclone was fixed as 0.5 mg mL^{-1}.

3.4. Method validation

According to the ICH guidelines the method was validated in terms of following parameters (ICH Q2R1 2005; USP 2008).

3.4.1. Limit of detection and limit of quantitation

The limit of detection for *R*-zopiclone was found to be 0.12 $\mu g.mL^{-1}$ for 10 μL of injection volume. The limit of quantitation for *R*-zopiclone was found to be 0.40 $\mu g.mL^{-1}$ for 10 μL of injection volume.

3.4.2. precision

The precision was evaluated by calculating the relative standard deviation (RSD%) of six determinations by injecting six freshly prepared solutions containing *R*-zopiclone at three concentration levels viz. 0.40, 2 and 5 $\mu g.mL^{-1}$ on the same day and the %RSD values were found to be 4.6, 3.1 and 3.9 respectively. For intra-day precision, the sample at the above three concentration levels was injected on six different days and the %RSD values were found to be 5.2, 3.8 and 4.3 respectively. The low RSD% values confirm the good precision of the developed method.

3.4.3. Linearity

The calibration curve was drawn between the peak areas of *R*-zopiclone versus its concentration in the range of 0.4 to 5 $\mu g.mL^{-1}$ of *R*-zopiclone. The coefficient of regression of the calibration curve was found to be 0.998, revealing that an excellent correlation existed between the peak area and concentration of the *R*-zopiclone.

3.4.4. Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the method. The accuracy was determined by spiking *R*-zopiclone to *S*-zopiclone at 0.4 (LOQ), 2 and 5 $\mu g.mL^{-1}$ levels with the three batches of *S*-zopiclone. Each determination was carried out in three times. Recovery of the *R*-zopiclone was 97.9, 97.3 and 99.8%, respectively.

3.4.5. Ruggedness and robustness

The standard addition and recovery experiments of *R*-zopiclone were carried out in *S*-zopiclone bulk samples at the same concentration levels tested in Laboratory A were again carried out at laboratory B using a different instrument by a different analyst. The data obtained from laboratory B was well in agreement with the results obtained in laboratory A, thus proving the method ruggedness.

In the varied chromatographic conditions like flow rate, mobile phase ratio, injection volume, UV wavelength and column temperature, the resolution between the peaks of *R* and *S*-enantiomers of zopiclone was found to be > 1.4 illustrating the robustness of the method.

3.5. Selectivity

Selectivity of the developed chiral reversed phase HPLC method was determined in presence of Imp-1 and Imp-2. Method selectivity was also challenged by forced degradation of *R*-zopiclone sample under and heat (60 °C) for 10 days and photo stability (not less than 1.2 million lux hours) studies. Content of *R*-zopiclone was checked in *S*-zopiclone sample exposed under light and heat on each day during the study period. *S*-Zopiclone sample did not cause any degradation and change in the *R*-zopiclone content during the study period. The exposed samples and both enantiomers of zopiclone samples peak homogeneity was confirmed by diode array detector.

3.6. Solution stability and mobile phase stability

Solution stability was studied by keeping the test solution in tightly capped volumetric flask at room temperature on a laboratory bench for 24 h. Content of *R*-zopiclone was checked for every 6 h interval and compared with freshly prepared solution. No variation was observed in the content of *R*-zopiclone for the study period and this indicates *S*-zopiclone sample solutions prepared in diluent were stable up to 24 h at room temperature.

Mobile phase stability was carried out by evaluating the content of *R*-zopiclone in *S*-zopiclone sample solutions, which were prepared freshly at every 6 h interval for 24 h. The same mobile phase was used during the study period. No variation was observed in the content of *R*-zopiclone for the study period and it indicates prepared mobile phase was found to be stable up to 24 h at room temperature.

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