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Enantiomeric separation of 1, 2, 3, 4-tetrahydro-1-naphthoic acid using chiral stationary Phase

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ABSTRACT

A new simple isocratic chiral liquid chromatographic method was developed for the enantiomeric purity of 1, 2, 3, 4-tetrahydro-1-naphthoic acid (THNA), a key starting raw material for the synthesis of Palonosetron HCl final drug substance. The chromatographic separation was achieved on Chiralcel OD-H, 250 mm X 4.6 mm, 5 µm column using a mobile phase system consisting of n-hexane, isopropyl alcohol and trifluoroacetic acid in the ratio of 948:50:2 (v/v/v). The mobile phase was pumped through column at the flow rate of 0.8 mL min^{-1}. Addition of trifluoroacetic acid in the mobile phase enhanced chromatographic efficiency and resolution between the isomers. The resolution between the isomers was found to be more than three. The developed method was subsequently validated and proved to be an accurate, specific and precise. The experimentally established limit of detection and quantification for (R)-enantiomer were found to be 0.4046µg mL^{-1} and 0.8186 µg mL^{-1} respectively and for (S)-THNA were found to be 0.5916µg mL^{-1} and 0.9860 µg mL^{-1} respectively for 20 µl injection volumes. The percentage recoveries of (R)-enantiomer were ranged from 98 to 102. The stability of sample in analytical solution was checked for about 36 hours at room temperature and was found to be stable for about 36 hours. The proposed method was found to be suitable and accurate for the quantitative determination of (R)-enantiomer of THNA in (S)-THNA.

Keywords: 1, 2, 3, 4-tetrahydro-1-naphthoic acid (THNA), Chiral Purity, Normal Phase, Development, Validation.

INTRODUCTION

1, 2, 3, 4-tetrahydro-1-naphthoic acid (THNA) is a key raw material in the synthesis of Palonosetron HCl. Palonosetron Hydrochloride Is an Effective and Safe Option to Prevent Chemotherapy-induced Nausea and Vomiting in Children [1] and also Palonosetron triggers 5-HT_3 receptor internalization and causes prolonged inhibition of receptor function [2].

Enantiomeric purity of chiral drug candidates has become very important particularly in the pharmaceutical and biological fields, because few enantiomers of racemic drugs have relatively different pharmacokinetic properties and diverse pharmacological or toxicological effects [3-6].

Enantiomeric separations have acquired importance in all stages of drug development and commercialization process. Therefore, the development of new methods for efficient chiral separations mainly based on HPLC, capillary electrophoresis (CE) or gas chromatography (GC) is more than necessary. The chromatographic separation of

enantiomers using high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) is one of the most useful and popular techniques for enantiopurity analysis in pharmaceutical preparations and biological fluids [7-9].

The thorough literature survey revealed that none of the most recognized pharmacopoeias or any journals includes this compound under investigation for the determination chiral purity. However few articles are reported for the determination of Palonosetron using LC-MS [10-12] and enantioseparation using micellar electrokinetic chromatography [13]. The chiral purity of THNA (figure – 1a), a key raw material used in the synthesis of Palonosetron (Figure - 1b) is very important to control the chiral purity of final Active Pharmaceutical Ingredient. Hence it is desirable to develop a liquid chromatographic method for the determination of chiral purity of THNA, which serves a reliable, sensitive and accurate.

(a) **(b)**

Figure 1: (a) (S)-1, 2, 3, 4-tetrahydro-1-naphthoic acid (THNA) (b) Palonosetron HCl from

MATERIALS AND METHODS

2.1. Chemicals

1, 2, 3, 4-tetrahydro-1-naphthoic acid (THAN) working standards and its respective (R)-enantiomers were kindly supplied by Research and Development department of Wockhardt Limited, Aurangabad, India, and the chemical structure is shown in figure-1. HPLC grade n-hexane, isopropyl alcohol and isopropyl alcohol were purchased from Merck Ltd, India. Laboratory reagent grade trifluoroacetic acid was purchased from Merck Ltd, India.

2.2. Equipment

A Waters 2695 separation module (USA) coupled to ultraviolet detector equipped with an auto injector and thermostated column oven compartment was utilized for method development and validation. Empower software was used for data acquisition and system suitability calculations.

2.3. Solution preparation

2.3.1. Preparation of system suitability solution

A solution containing a mixture of 10µg mL^{-1} of R-THNA and 1000µg mL^{-1} of S-THNA was prepared in n-hexane and ethanol in the ratio of 95:5 (v/v).

2.3.3. Preparation of standard solution

A solution containing 10µg mL^{-1} of R-THNA impurity standard was prepared in n-hexane and ethanol in the ratio of 95:5 (v/v).

2.3.4. Preparation of sample solution

A solution containing 1000µg mL^{-1} of S-THNA sample was prepared in n-hexane and ethanol in the ratio of 95:5 (v/v).

2.4. Chromatographic conditions

The chromatographic conditions were optimized using chiral stationary phase Chiralcel OD-H, 250 mm X 4.6 mm, 5µm, (Daicel). The mobile phase consisting of n-hexane, ethanol and trifluoro acetic acid in the ratio of 948:50:2 (v/v/v) was pumped through the column at the flow rate of 0.8 ml min^{-1}. The column oven compartment was maintained at 30°C and the detection was carried out at a wavelength of 265 nm. The injection volume was 20µl.

RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

The aim of the work is to separate the enantiomers for the accurate quantification of (R)-enantiomer. A solution containing 1 mg mL^{-1} of (S)-THNA and 0.01 mg mL^{-1} of R-isomer prepared in mobile phase was used in the method development. To develop a rugged and suitable LC method for the separation of enantiomers, different mobile phases and stationary phases were employed. To achieve the chromatographic separation various chiral columns like Chiral AGP, YMC Chiral NEA (S), Chiralcel OD-RH, Chiralcel OD-H and Chiralpak AD-H were used. Various experiments were conducted, to select the best stationary and mobile phases that would give optimum resolution and selectivity for the enantiomers. But satisfactory separation was not found in all above experiments. There is an indication of separation on Chiralcel OD-H column using a mobile phase consisting of n-hexane, ethanol in the ratio of 950:50 (v/v) but the peak shapes were found broad and poor resolution. Introduction of trifluoroacetic acid in the mobile phase improved the chromatographic efficiency and resolution between the enantiomers. A satisfactory separation was achieved on Chiralcel OD-H column (resolution between enantiomers was found greater than 3) using the mobile phase system n-hexane, ethanol, trifloroacetic acid in the ratio of 948:50:2 (v/v/v) (Figure – 2). Due to the better chromatographic results obtained on the Chiralcel OD-H column, the method validation was carried out on the same column. In the optimized method, the typical retention times of (S)-THNA and its (R)-enantiomer were found about 7.6 min and 8.8 min, respectively. Hence it is very easy to check the chiral purity at starting stage of synthesis. The typical chromatogram of (S)-THNA spiked with its R-Isomers is shown in figure – 2. The system suitability test results of the chiral LC method are presented in Table 1.

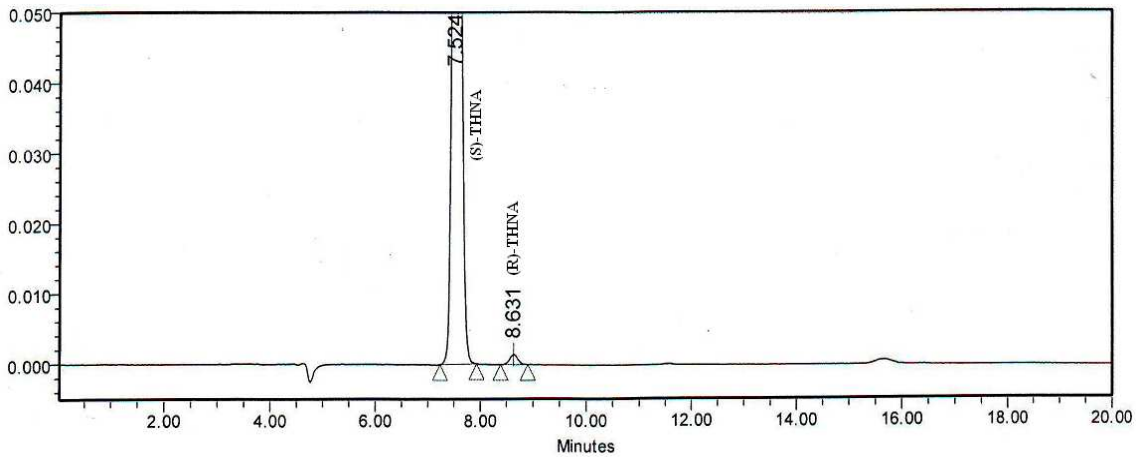

Figure 2: Typical HPLC chromatograms of chiral purity of S-THAN spiked with R-THAN

3.2. Method validation

The optimized chiral purity method for S-THNA was validated according to ICH guidelines [14], with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability parameters were also assessed.

3.2.1.System suitability test

The system suitability test was performed according to USP 30 [15] and BP 2007 [16] indications. The observed RSD values at 1% level of analyte concentration were well within the usually accepted values ($\leq$2%). Theoretical plates (N), USP tailing factor (T_f) and USP resolution (R_s) between S-THNA and R-isomer were also determined. The results obtained were all within acceptable limits (Table-1).

Table 1: System suitability parameters

Analyte (n=6)	Tailling factor (T_f)[a]	Efficiency (plates m-1)[a]	Resolution (R_s)[a]	RSD (%)
R-THNA	1.07	14423	4.12	0.64

a calculated according to the USP.

3.2.2. Specificity

The specificity of the method was checked by injecting blank solution and sample solution. There was no interference from blank at the retention time of analyte peak. Specificity was also checked by exposing the sample under stressed conditions like heat (50°C) for 12 hrs, light (1.2 million lux) for 22 hrs, humidity (40°C/75%RH) for 7 days. The results are tabulated in table 2.

Table 2: Results of stress study

Condition	Conditions	Time	Temperature (°C)	%Degradation
Thermal	-	12Hrs	50°C	4.19
Photolytic	250 watt h/m^2	22Hrs	-	1.56
Humidity	40°C/75% RH	8 days	-	1.01

The peak purity indices for the analytes in stressed sample and spiked sample were determined with PDA detector under optimized chromatographic conditions found to be better (purity angle < purity threshold) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference. The typical HPLC chromatograms of stressed conditions are represented in Figure – 3.

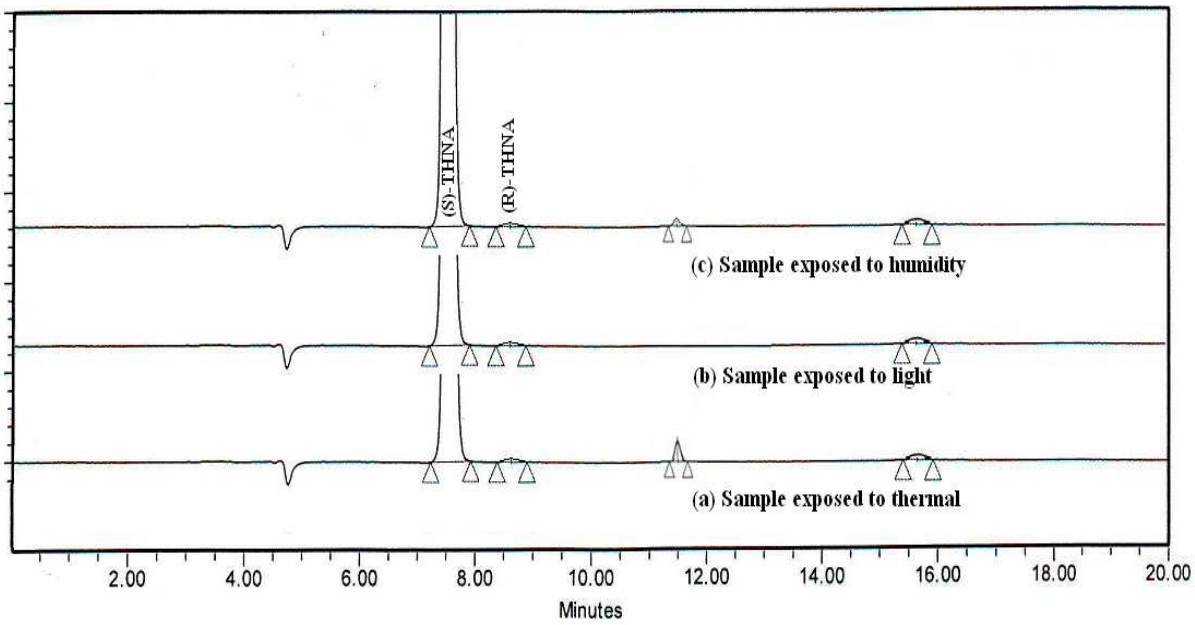

Figure 3: Typical HPLC chromatograms of stressed samples (a) Sample exposed to thermal, (b) Sample exposed to humiditylight and (c) Sample exposed to humidity.

The stability of drugs in analytical solution was checked by preparing sample solution as per method and injected at regular time intervals in the proposed method at room temperature. Verified the formation of additional peaks and found that no additional peaks were formed till 36 hours indicating that the sample solution is stable for about 36 hours at room temperature.

3.2.3.Method precision and ruggedness

Method reproducibility was determined by measuring repeatability and intermediate precision of peak area counts of each enantiomer. In order to determine the repeatability of the method, replicate injection (n=6) of a solution containing 1 mg mL of S-THNA spiked with (R)-enantiomer (1%) was carried out. The intermediate precision was also evaluated on two different days by performing six successive injections each day. The results are tabulated in table 3.

3.2.4.Linearity and range

The nominal concentration of S-THNA in test solution was 1 mg ml^{-1}. Taking into account that typical impurity tolerance levels is 1 % for R-isomer and response function was determined by preparing standard solution of R-isomer at different concentration levels ranging from lower limit of quantification to 150 % of impurity tolerance level and that identification of impurities below lower level of quantification were not considered to be necessary unless the potential impurities are expected to be unusually potent to toxic.

The plots of area under the curve (AUC) of the peak responses of the analytes against their corresponding concentrations, they fitted straight lines responding to equations. The y-intercepts were close to zero with their confidence intervals containing the origin. The correlation coefficients (r) were exceeds 0.98, the acceptance threshold suggested for linearity of procedures for the determination of impurity content in bulk drug [17]. Furthermore the plot of residuals exhibited random patterns with the residuals passing the normal distribution test ($p < 0.05$), all of which evidenced that the method is linear in the tested range. The regression statistics are shown in table 3.

Table – 3: Validation results of precision, Regression statistics, LOD and LOQ

Validation parameters	Results
Repeatibility (n=6, %RSD)	
Retention time (S)-enantiomer	0.67
Retention time (R)-enantiomer	0.87
Peak area (R)-enantiomer	1.33
% w/w of (R)-enantiomer	2.34
Intermediate Precision (n=12, %RSD)	
Retention time (S)-enantiomer	0.56
Retention time (R)-enantiomer	0.46
Peak area (S)-enantiomer	0.97
% w/w of (S)-enantiomer	2.91
Regression statistics (R-enantiomer)	
Calibration range (µg/mL)	0.8092 µg mL^{-1} to
t-stat	15.1725
P-value	801.99
95% confidence interval (Lower)	1.68E-44
95% confidence interval (Upper)	2990.17
Correlation coefficient	3005.82
LOD-LOQ	0.99999
Limit of detection (µg/mL) (R-THAN)	
Limit of quantification (µg/mL) (R-THAN)	0.4046
Limit of detection (µg/mL) (S-THAN)	0.8186
Limit of quantification (µg/mL) (S-THAN)	0.5916
	0.9860

3.2.5.Determination of limit of quantification and detection (LOQ and LOD)

The linearity performed above, used for the determination of limit of quantification and detection. Residual standard deviation (σ) method was applied and were predicted the LOQ and LOD values using following formula (a), (b) and established the precision at these predicted levels. The results are tabulated in table-3

$$LOQ = 10\sigma / S \qquad (a)$$

$$LOD = 3.3\sigma / S \qquad (b)$$

Where σ = Residual standard deviation of response, S = Slope of the calibration curve

3.2.6.Accuracy

Accuracy was evaluated by the determination of R-isomer in solution prepared by standard addition method. The experiment was carried out by adding known amount of R-isomer corresponding to three concentration levels of 40 %, 100 % and 150 % of the impurity tolerance level in sample solution. The samples were prepared in triplicate at each level. The quantification of added analyte (% weight/weight) was carried out by using an external standard of S-isomer prepared at the analytical concentration. The experimental results revealed that approximate 95 - 105 % recoveries were obtained for R-isomer in drug substance. Therefore, based on the recovery data (Table - 4) the

estimation of R-isomer that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis.

Table – 4: Recovery Data

Spike Level	Amount added* (µg/mL)	Amount recovered* (µg/mL)	Mean Recovery* (%)	RSD* (%)
40	4.238	4.186	98.77	3.46
100	9.865	10.046	101.83	2.36
150	15.026	14.898	99.15	2.88

* n=3

CONCLUSION

A simple, rapid and accurate normal phase chiral LC method was described for the enantiomeric separation of S-THNA a starting material of Palonosetron HCl. The method was validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the quantitative determination of chiral impurity ((R)-enantiomer) in S-THNA.

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