GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

# Method development and validation of taraxerol by using high performance liquid chromatography

Priyanka D. Gore *, Vikas Rajurkar, Sajid Shaikh, Karna B Khavane and Pallavi Patharkar

*Dr. Vedprakash Patil Pharmacy College Gevrai Tanda, Paithan Road, Chh. Sambhajinagar,Maharashtra, India.*

GSC Biological and Pharmaceutical Sciences, 2024, 28(03), 180–192

Publication history: Received on 08 August 2024; revised on 14 September 2024; accepted on 17 September 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.3.0332

## Abstract

Taraxerol is a bioactive compound found in several higher plants that exhibits many specific biological effects, particularly in the field of medicine. Although taraxerol has limited antioxidative efficacy and only modest antibacterial activities, many studies have shown its promise as an anti-plasmodial, antidiabetic, anticancer, anti-inflammatory, and anti-dermatophyte agent. A robust analytical approach for quantifying Taraxerol using High Performance Liquid Chromatography (HPLC) is the main objective of this research endeavor. An analysis of Taraxerol revealed a retention time of 3.519 minutes. Within the concentration range of 20-70 µg/mL, the drug exhibited a linear relationship, with a correlation coefficient of 0.999. The determination of the limit of detection (LOD) and limit of quantitation (LOQ) yielded values of 0.0026 µg/mL and 0.0081 µg/mL each. The accuracy of the approach was considered satisfactory, as the average recovery percentage was within the allowed range of 99.92-100.33%.

**Keywords:** RP-HPLC; Method development; Validation; Taraxerol

## 1. Introduction

The field of analytical chemistry is primarily concerned with the qualitative and quantitative characterization of matter composition. It encompasses the analysis of drug samples in bulk, pharmaceutical formulations, and biological fluids, using a wide range of analytical methods. Chromatography is an analytical method used to purify and separate organic and inorganic compounds [1].

The development of analytical techniques eventually results in the development of universally accepted test methods. Thus, quality control laboratories used these methods to confirm the efficacy, truthfulness, purity, safety, and performance of pharmaceutical products. Industrial regulatory authorities give priority to analytical processes in manufacturing. Regulatory agencies require that applicants seeking drug approval must show expertise in the whole drug development process using accepted analytical methods [2,3].

HPLC is an analytical method that resolves solutes by varying rates of elution as they flow through a chromatographic column. The separation process implemented by this instrument is determined by the distribution of the mobile phase and stationary phase [4]. Advanced HPLC analytical techniques, influenced by several factors, provide substantial volumes of data during analytical investigations. While HPLC is a dynamic separation method with many uses, the procedure may be occasionally challenging because of its many variables that must be carefully adjusted before each run [5].

There are many approaches now used in the development of HPLC methods [6]. The HPLC technique is suitable for analyzing most pharmaceuticals in multi component dosage forms because to its various benefits such as speed, specificity, accuracy, precision, and simplicity of automation. Development and validation of HPLC techniques are

* Corresponding author: Priyanka Gore

crucial in the fields of novel discovery, development, manufacturing of pharmacological therapies, and other investigations involving people and animals. A systematic analytical method is devised to evaluate a specific attribute of the drug ingredient or drug product in comparison to predetermined acceptance standards for those properties [7].

The analytical procedures used for herbal analysis must be cost-effective, rapid, and generate minimal amounts of volatile chemical waste. Presently, the analytical community is interested in researching non-hazardous and environmentally friendly approaches to produce many green chromatographic methods for regular quality analysis. In order to address the expensive nature of phytochemical analysis and the use of dangerous chemicals in advanced equipment, an effort was undertaken to create an easy analytical technique for many samples that requires less time and solvents [8].

Taraxerol is a pentacylic triterpenoid with the chemical formula (3β)-D-Friedoolean-14-en-3-ol. In 1955, Beaton et al. determined that the chemical structure of oleanane-3-ol is characterized by the absence of a methyl group at position 14, the presence of an α-methyl substituent at position 13, and a double bond between positions 14 and 15 [9,10].

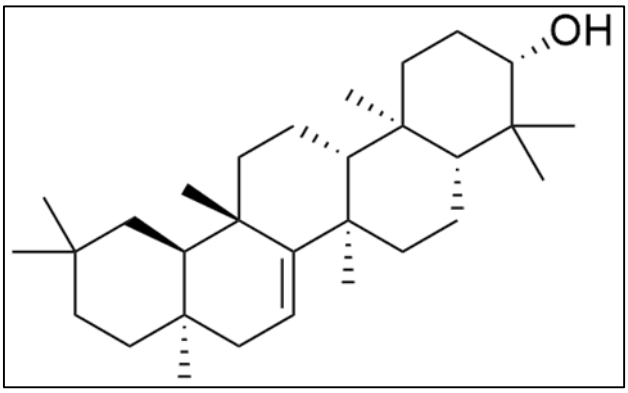


**Figure 1** Structure of Taraxerol

## 2. Materials and methods

In this work, the SHIMANDZU LC-2010 AHT HPLC system was used. The setup included a Khromasil 100-5-C8 250X4.6mm,5µm column, a pump, and a Lasany LI-2702 UV/VIS detector with adjustable wavelength configuration. The chromatographic data collection was conducted using Empower software version 2. The procedure of chromatographic separation was carried out using a Khromasil -C18 column.

### 2.1. Chemicals and Reagents

Materials and solvents used in the study were of analytical grade and met the criteria for analysis and HPLC.The proper use of high-quality reagents, solvents, and filters is essential to ensures the accuracy and reliability of the HPLC analysis.

#### *2.1.1. Preparation of standard stock solution*

A precisely measured amount of Taraxerol working standard, about 10 mg, was placed into a 10.0 mL volumetric flask. Some 5.0 mL of high-performance liquid chromatography (HPLC) grade methanol was added to the volumetric flask and subjected to sonication to dissolve the medication. The mixture was cooled to ambient temperature and then diluted with methanol (HPLC Grade) to the desired concentration, resulting in a final stock solution of 1000 µgm/ml of Taraxerol.

#### *2.1.2. Further Dilution (or ) Optimized method solutions Preparation*

The mobile phase was allowed to reach equilibrium with the stationary phase until a stable baseline was measured. A newly manufactured standard stock solution of Taraxerol was transferred into a 10ml volumetric flask and then diluted with methanol. A volume of 0.1, 0.2, 0.3, 0.4, and 0.6 mL of solution was transferred using a pipette into a 10 mL volumetric flask. The volume was then increased to 10 mL by adding mobile phase to get a final concentration of 10, 20, 30, 40, and 60µ-g/mL of Taraxerol. Samples were injected and peaks were detected at a wavelength of 276 nm.

*2.1.3. Method development selection of wavelength*

By scanning the standard solution of Taraxerol in the wavelength range of 200-400 nm on a UV-Visible Spectrophotometer, the wavelength at which maximum absorbance (μ max) occurred was determined to be 276 nm for Taraxerol. Therefore, the detection wavelength for the chromatographic investigation was selected as 276 nm. The spectrum is seen in Figure 2.

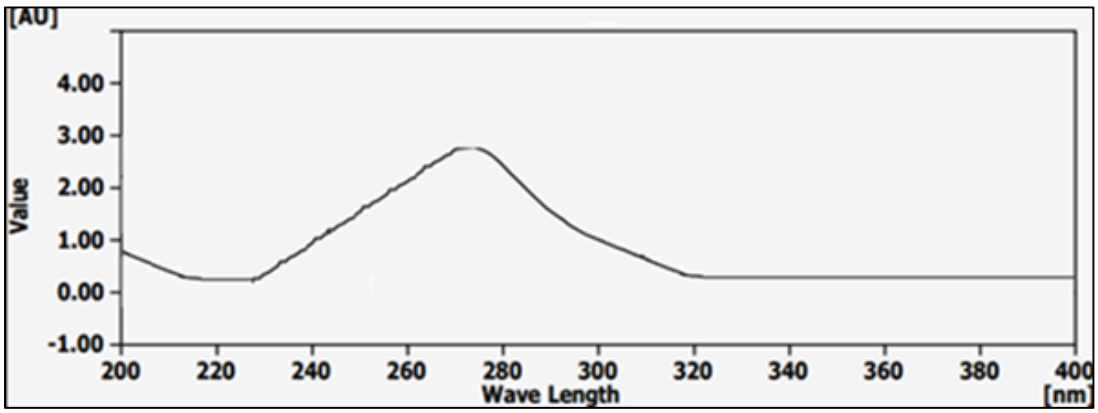


**Figure 2** UV spectrum of Taraxerol

## 3. Results and discussion

### 3.1. Chromatographic conditions

The parameters for the chromatographic conditions of the developed method were shown in table 1, along with the standard chromatogram of the drug shown in figure 3.

**Table 1** Chromatographic Conditions

| Sr. No. | Parameters | Method |
|---|---|---|
| | **Stationary phase (column)** | **C18 (Inertsil) (4.6 × 250mm, 5µm)** |
| 2. | Flow rate (ml/min) | ml/min |
| 3. | Mobile Phase | Methanol: Water (80:20)0.1%OPA |
| 4. | Column temperature (°C) | Ambient |
| 5. | Run time (minutes) | 10 min |
| 6. | Detection wavelength (nm) | 276.0 nm |
| 7. | Volume of injection loop (µl) | 20.0 µL |
| 8. | pH | 6.6 |

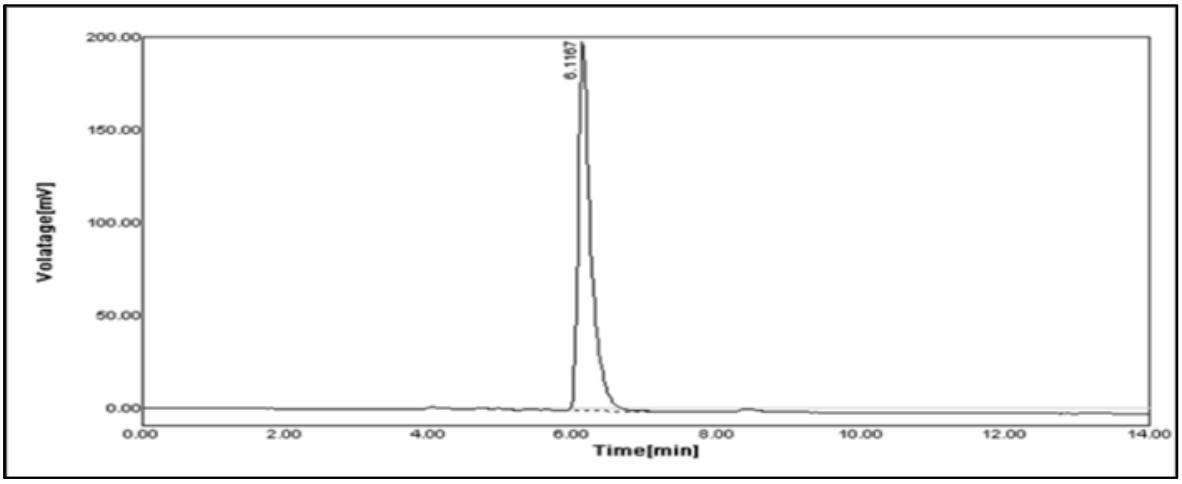


**Figure 3** Standard chromatogram for Taraxerol

### 3.2. Method validation

*3.2.1. System suitability*

Based on the data obtained, the system suitability parameters were seen to fall within the specified limits, indicating that the HPLC system was functioning well and generating reliable outcomes. The relative standard deviation (RSD) values of peak area and retention duration for medications were within a range of 2%, suggesting the system's suitability. The relevant findings may be found in table 2.

**Table 2** System suitability parameters for Taraxerol

| Sr. No | Concentration (µg/ml) | Peak area | Amount found | %Amount found |
|---|---|---|---|---|
| 1 | 10 | 2069.205 | 9.95 | 99.5 |
| 2 | 10 | 2071.554 | 9.96 | 99.6 |
| Mean | | 2070.38 | 9.96 | 99.55 |
| SD | | 1.66 | 0.01 | 0.07 |
| %RSD | | 0.08 | 0.07 | 0.07 |

*3.2.2. Linearity*

The calibration studies produced data that, when analyzed using linear regression, revealed a linear correlation between peak areas and concentrations throughout the range of 10-60µg/mL for Taraxerol. The correlation coefficient for the data was 0.9993. The findings of linearity are shown in Figure 4 and Table 3.

**Table 3 L**inearity value

| Sr. No | Concentration in µg/ml | Peak Area |
|---|---|---|
| 1 | 10 | 2069.036 |
| 2 | 20 | 4249.001 |
| 3 | 30 | 6155.832 |
| 4 | 40 | 8333.22 |
| 5 | 50 | 12530.58 |
| | Slope | 269.01 |

| | | |
|---|---|---|
| | Intercept | 1472.5 |
| | Correlation coefficient ($R^2$) | 0.9993 |

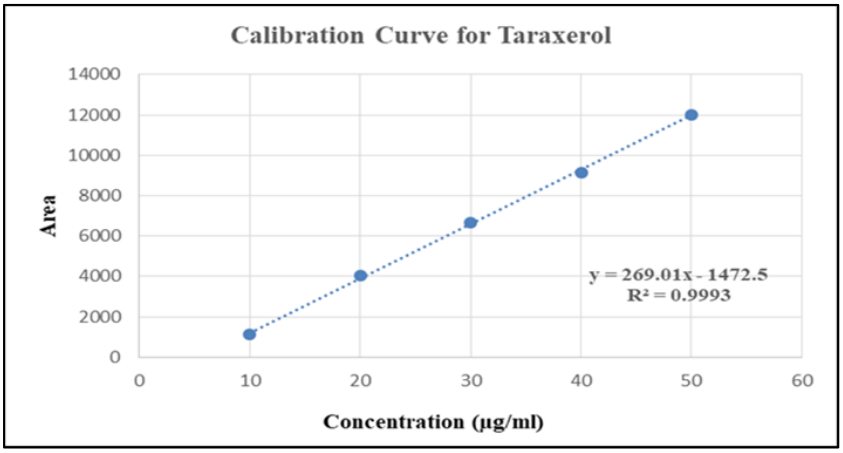


**Figure 4** Calibration curve for Taraxerol

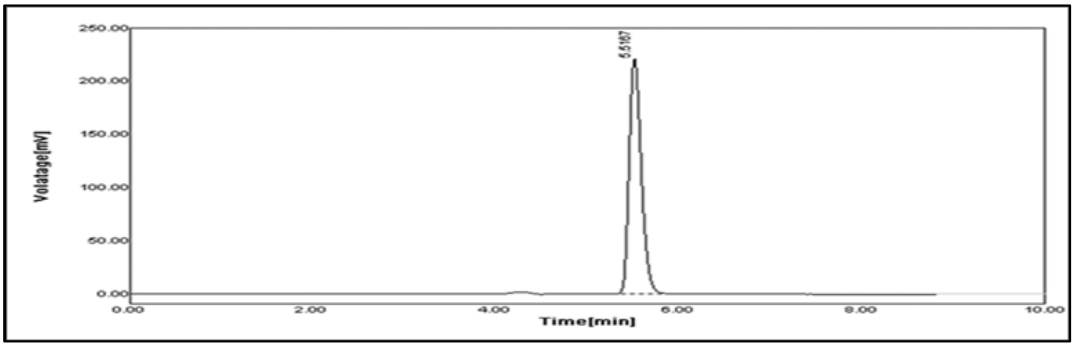


**Figure 5** Linearity chromatogram of Taraxerol for 10µ/mL

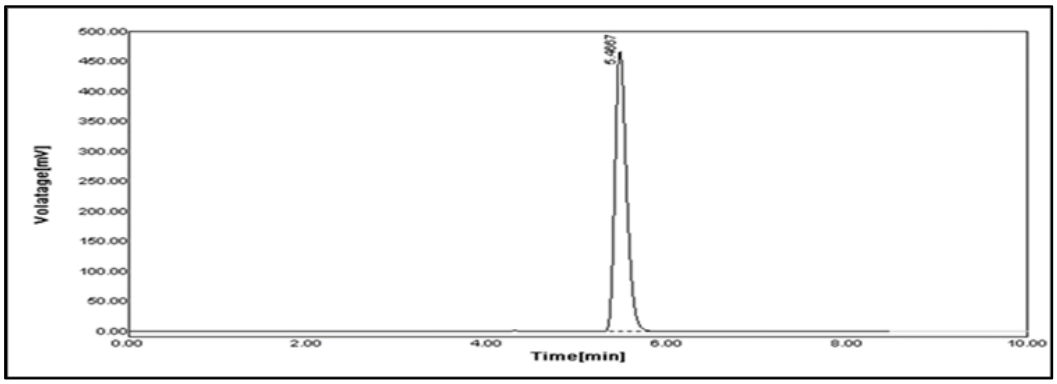


**Figure 6** Linearity chromatogram of Taraxerol for 20µg /mL

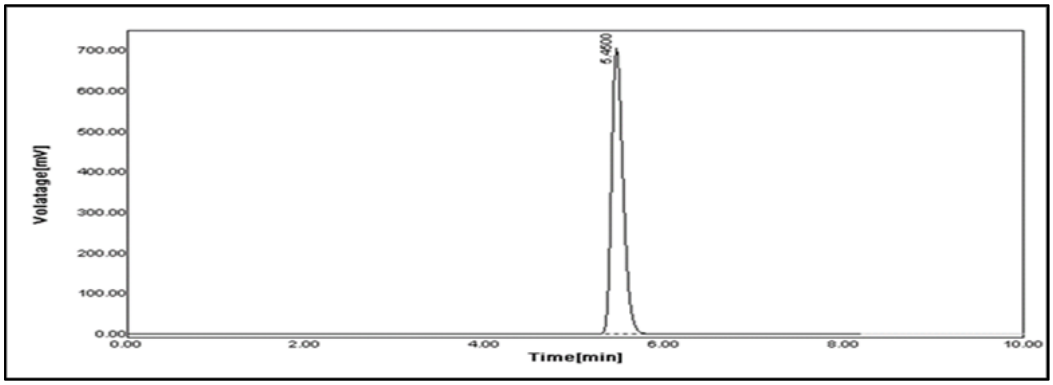


**Figure 7** Linearity chromatogram of Taraxerol for 30µg /mL

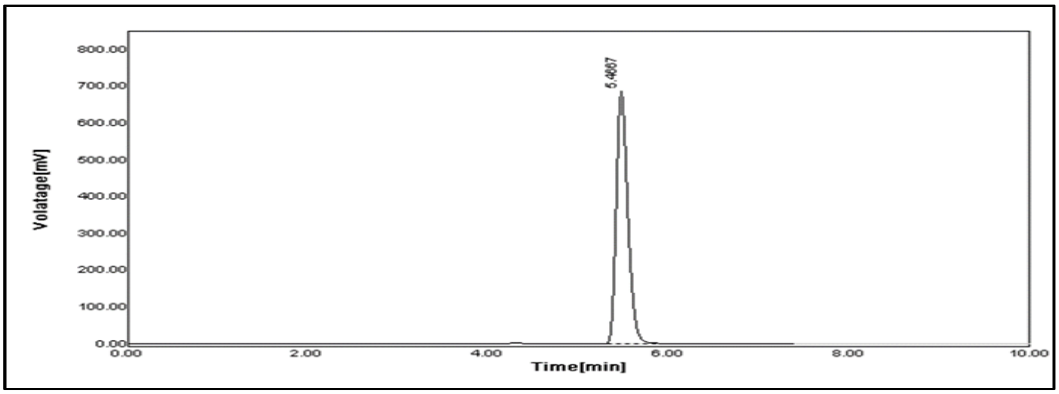


**Figure 8** Linearity chromatogram of Taraxerol for 40µg /mL

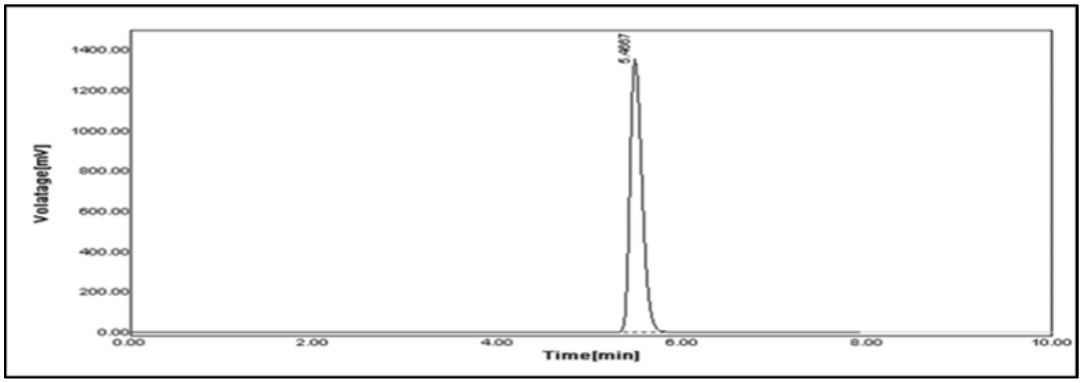


**Figure 9** Linearity chromatogram of Taraxerol for 50µg /mL

*3.2.3. Precision*

Precision of an analytical technique refers to the level of consistency among individual test findings when the method is subjected to repeated application on several samples of a uniform sample. Analytical technique accuracy is sometimes quantified by the standard deviation or relative standard deviation (Coefficient of variation) of a set of measurements. Analysis of the sample solution was conducted by injecting it three times (in duplicate) into the HPLC and recording the resulting chromatogram. The standard deviation, percentage relative standard (%RSD), and percentage relative mean error were computed for the measured values of retention time and peak area. An analysis of many duplicate standards of Taraxerol was used to develop the procedure. All the solutions were evaluated three times (in duplicate) to record any variations in the final result within a single day and between different days. Results of intraday and interday

precision investigations on the RP-HPLC technique for Taraxerol showed a high level of precision ranging from 98% to 100% for the analytical method. Figure 11 to figure 13 depicted the chromatogram for precision. The findings, as shown in Table 5 and 6, validate the dependability and consistency of the HPLC technique for analyzing Taraxerol.

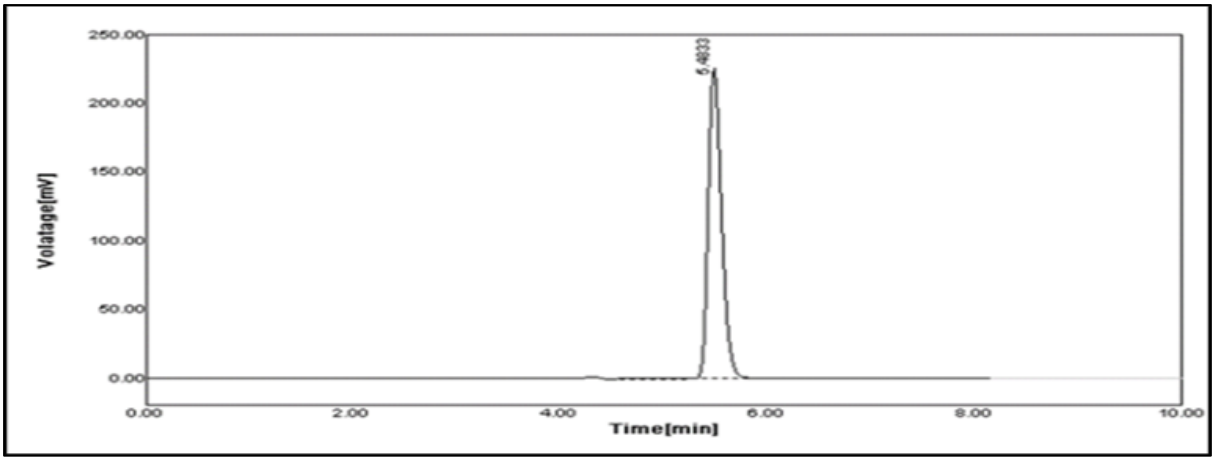


**Figure 10** Chromatogram of Taraxerol for precision 10µg /mL

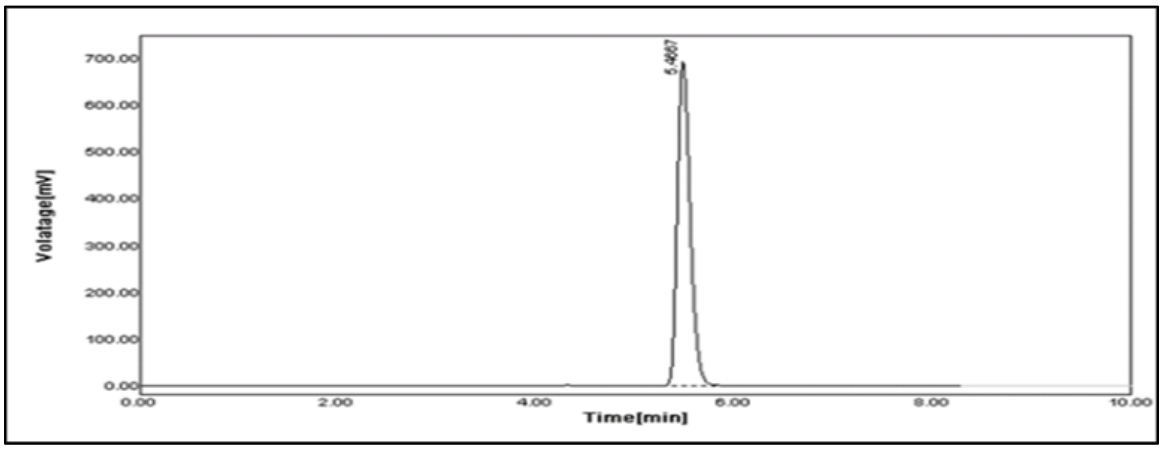


**Figure 11** Chromatogram of Taraxerol for precision 30µg /mL

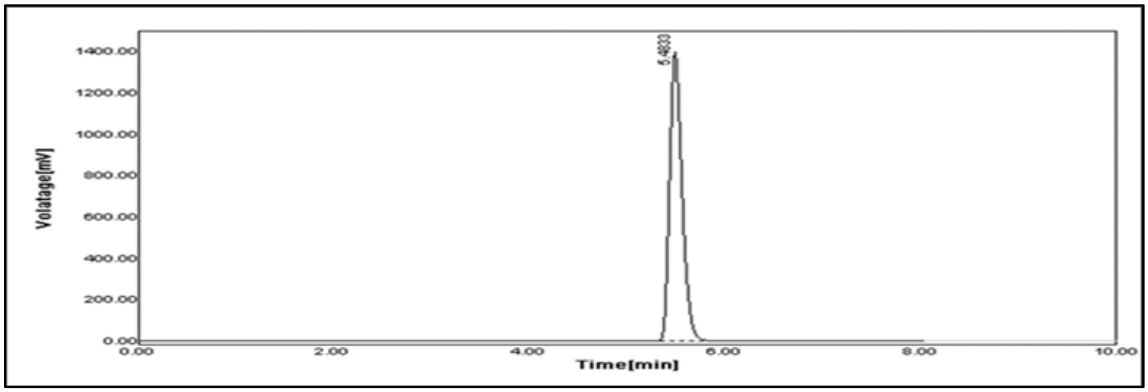


**Figure 12** Chromatogram of Taraxerol for precision 50µg /mL

**Table 5** Result of Intraday Precision studies on HPLC method for Taraxerol

| Sr. No | Conc. | Area I | Area II | Mean | Amount Found [µg/ml] | %Amount Found [µg/ml] | SD | %RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 2060.099 | 2036.254 | 2048.18 | 9.85 | 98.50 | 16.86 | 0.82 |
| 2 | 30 | 6127.815 | 6126.913 | 6127.36 | 29.38 | 97.93 | 0.64 | 0.01 |
| 3 | 50 | 12417.42 | 12412.68 | 12415.05 | 59.57 | 99.28 | 3.36 | 0.03 |

**Table 6** Result of Interday Precision studies on HPLC method for Taraxerol

| Sr. No. | Conc. | Area I | Area II | Mean | Amount Found [µg/ml] | %Amount Found [µg/ml] | SD | %RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 2051.146 | 2052.365 | 2052.37 | 9.87 | 98.70 | 0.86 | 0.04 |
| 2 | 30 | 6150.149 | 6148.253 | 6149.20 | 29.52 | 98.40 | 1.34 | 0.02 |
| 3 | 50 | 12418.57 | 12519.24 | 12468.90 | 59.83 | 99.72 | 71.18 | 0.57 |

*3.2.4. Accuracy or recovery studies*

Table 7 represents a summary of the data collected from the recovery studies, including the spiked concentrations and the corresponding average recovery percentages. Figure 13 to figures 15 are the chromatogram obtained for accuracy study.

**Table 7** Result showing percent recovery

| Sr.No | Level | Amount added (µg/mL) | Mean | % Recovery |
|---|---|---|---|---|
| 1. | 50 | 20 | 99.92 | 99.92 |
| 2. | 100 | 40 | 100.22 | 100.22 |
| 3. | 150 | 60 | 100.33 | 100.33 |

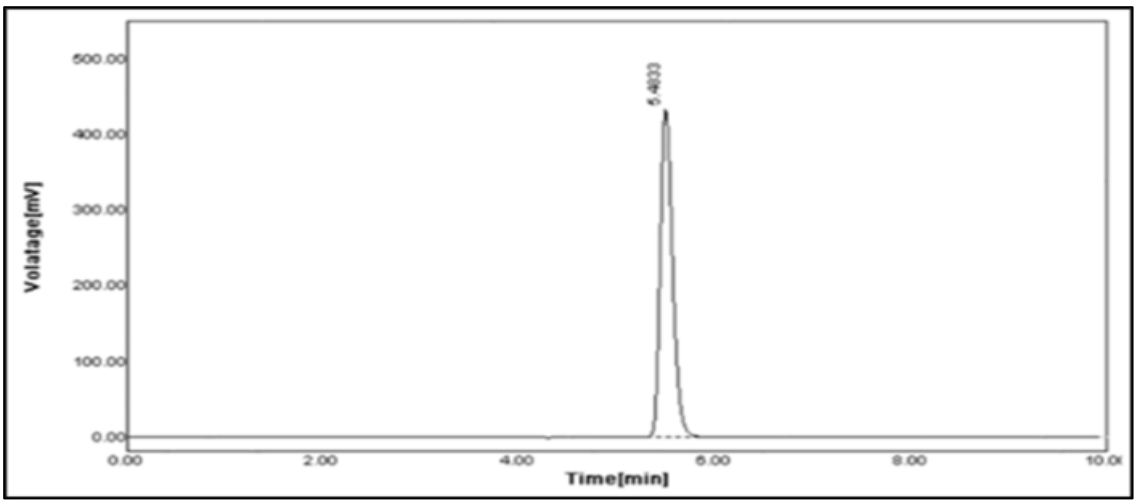


**Figure 13** RP-HPLC chromatogram of Taraxerol Accuracy at 80% level

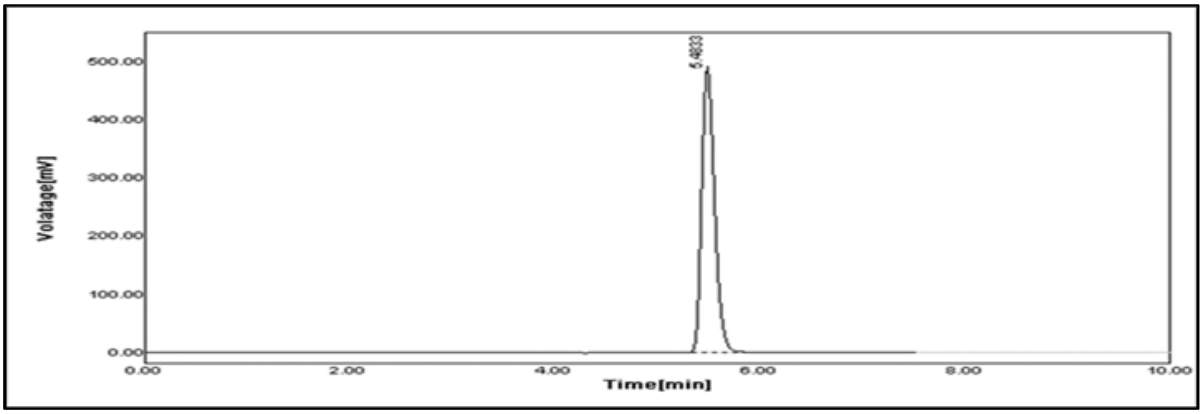


**Figure 14** RP-HPLC chromatogram of Taraxerol Accuracy at 100% level (100%)

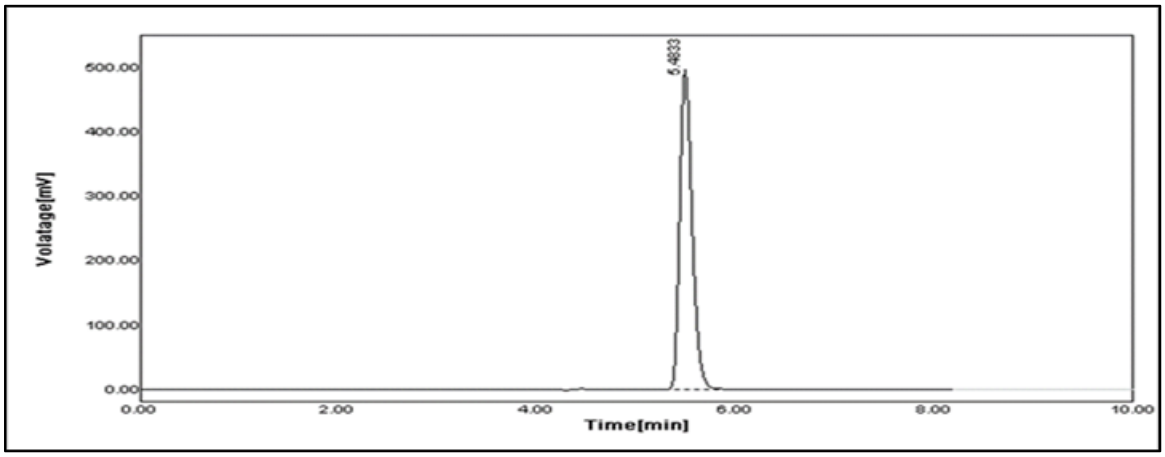


**Figure 15** RP-HPLC chromatogram of Taraxerol Accuracy at 120% level (120%)

*3.2.5. Limit of Detection (LOD)*

The method's LOD was determined to be 0.157 µg/ml.

*3.2.6. Limit of Quantification (LOQ)*

The method's LOQ was determined to be 1.116 µg/ml.

*3.2.7. Robustness*

**Table 8** Results of Robustness for Taraxerol

| **Change in flow** | | | | |
|---|---|---|---|---|
| **0.6mL flow** | | | **0.8 mL flow** | |
| **Sr.no** | **Conc(µgm/mL)** | **Area** | **Conc(µgm/mL)** | **Area** |
| 1 | 20 | 4405.56 | 20 | 3502.86 |
| 2 | 20 | 4518.61 | 20 | 3541.93 |
| | Mean | 4462.09 | Mean | 3522.40 |
| | SD | 79.94 | SD | 27.63 |
| | %RSD | 1.79 | %RSD | 0.78 |

Robustness of a technique refers to its capacity to maintain its performance even when subjected to incremental intentional changes in parameters. The robustness of the suggested technique was assessed by deliberately making modest adjustments to the optimum method parameters. This study investigated the impact of variations in the composition and flow rate of the mobile phase, as well as the wavelength, on the retention period and tailing factor of the drug peak. Optimal chromatographic conditions were achieved by varying the mobile phase composition in a percentage of ±1 ml/min-1, the flow rate by ±1 ml/min-1, and the wavelength change by ±1 ml/min-1. Since the robustness parameters were deemed acceptable, the analytical approach may be completed.

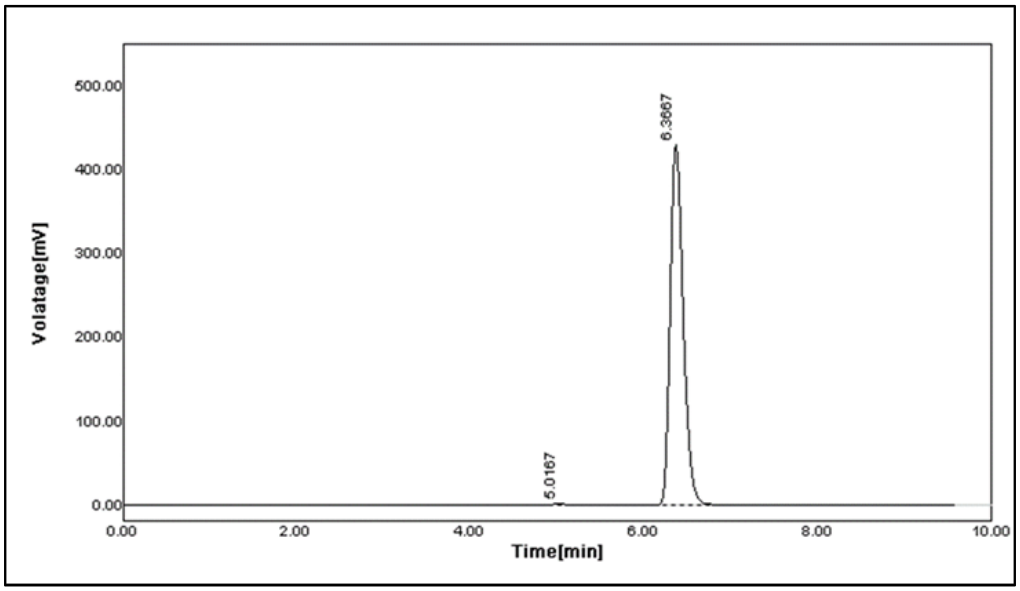


**Figure 16** Change in Flow rate

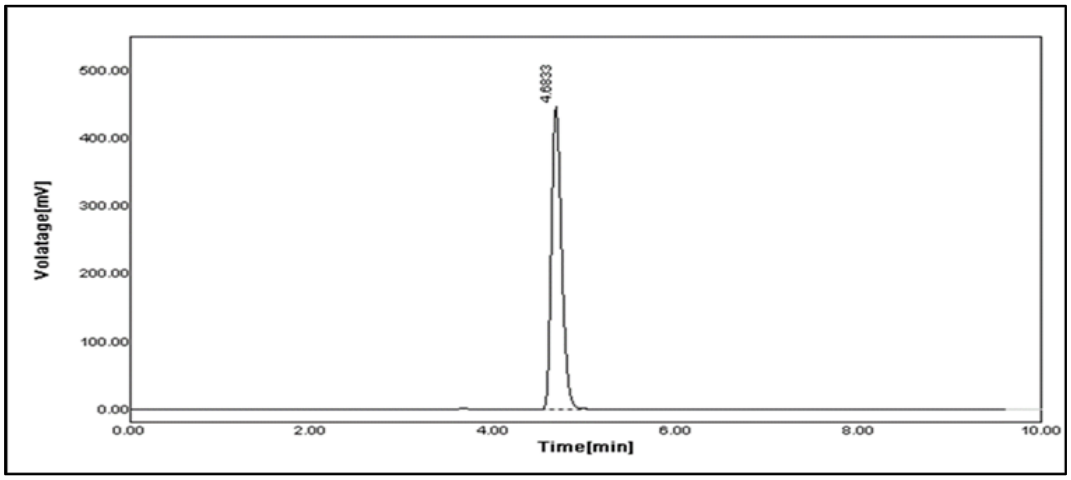


**Figure 17** Chromatogram of Taraxerol for Robustness flow rate (0.8ml)

**Table 9** Data of Taraxerol Robustness with change in mobile phase composition

| **Change in Mobile Phase** | | | | |
|---|---|---|---|---|
| **79M:21H2O** | | | **81M:19H2O** | |
| **Sr.no** | **Conc(µgm/mL)** | **Area** | **Conc(µgm/mL)** | **Area** |
| 1 | 20 | 3401.27 | 20 | 3613.6 |
| 2 | 20 | 3435.31 | 20 | 3535.3 |
| | Mean | 3418.3 | Mean | 3574.45 |
| | SD | 24.07 | SD | 55.37 |
| | %RSD | 0.70 | %RSD | 1.55 |

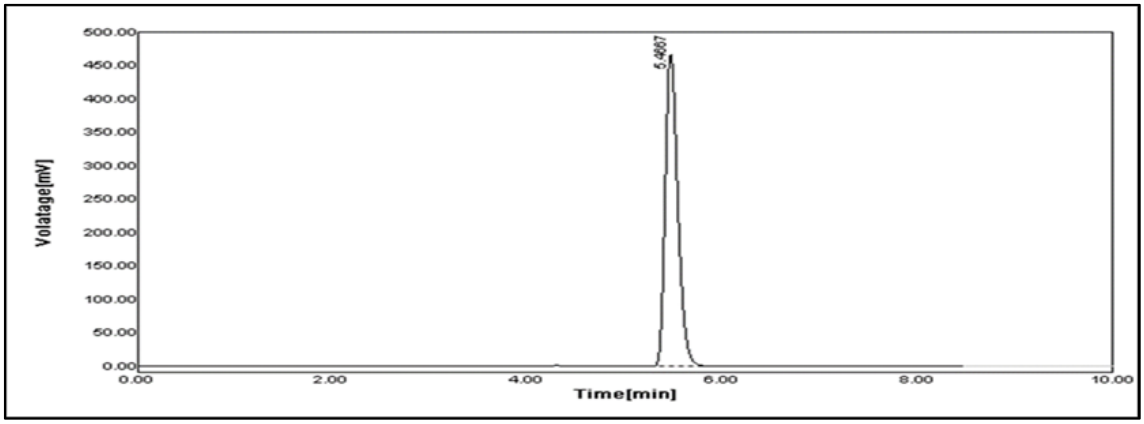


**Figure 18** RP-HPLC chromatogram of Taraxerol for mobile phase Methanol: Water 0.1%OPA (79:21)

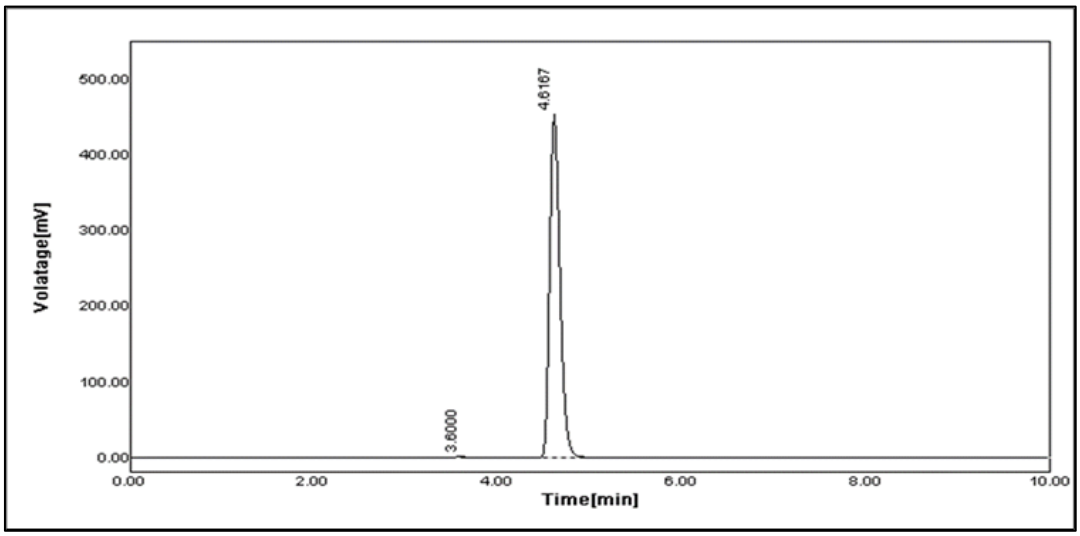


**Figure 19** RP-HPLC chromatogram of Taraxerol for mobile phase Methanol: Water 0.1%OPA (81:19)

**Table 10** Data of Taraxerol Robustness with change in Wavelength

| **Change in Wavelength** | | | | |
|---|---|---|---|---|
| **276 nm** | | | **278 nm** | |
| **Sr.no** | **Conc(µgm/mL)** | **Area** | **Conc (µgm/mL)** | **Area** |
| 1 | 20 | 4126.11 | 20 | 4139.07 |
| 2 | 20 | 4135.99 | 20 | 4149 |
| | Mean | 4131.1 | Mean | 4144.04 |
| | SD | 6.99 | SD | 7.02 |
| | %RSD | 0.17 | %RSD | 0.17 |

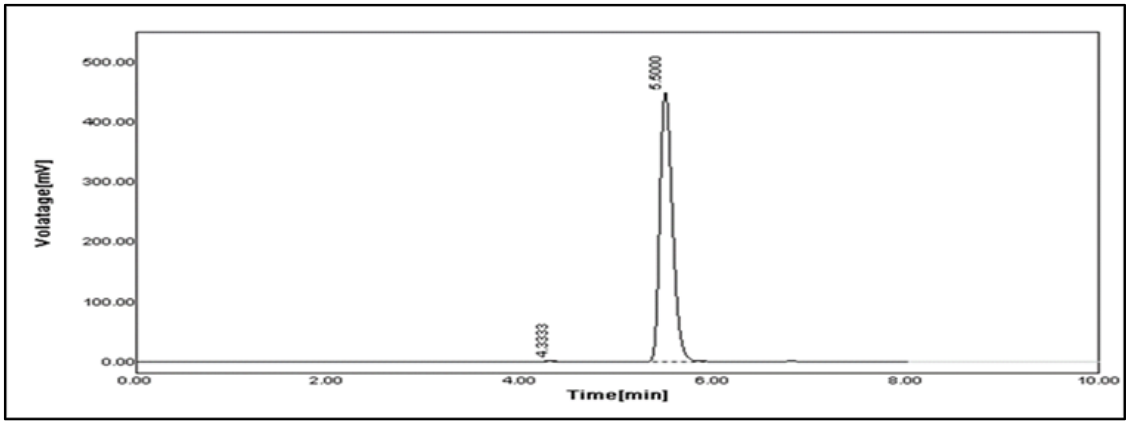


**Figure 20** Chromatogram of Robustness change in wavelength 274 nm

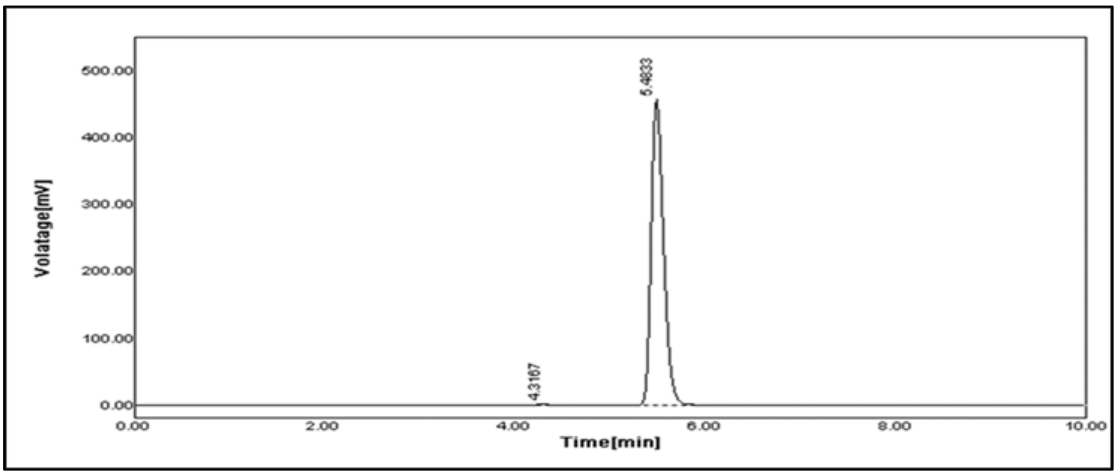


**Figure 21** Chromatogram of Robustness change in wavelength 278 nm

*3.2.8. Ruggedness*

Ruggedness study of drug was carried out at the three different temperature levels. From the results it was found that the method was rugged showing the % RSD value less than 2%.

**Table 11** Evaluation data for Ruggedness of Taraxerol

| **Taraxerol** | | | |
|---|---|---|---|
| **Concentration (µg/ml)** | **Temperature (°C)** | **Absorbance** | **% RSD** |
| 8 | 25 | 0.818 | 0.560 |
| 10 | 37 | 0.991 | 0.202 |
| 12 | 60 | 1.140 | 0.450 |

## 4. Conclusion

The proposed method has been validated to evaluate its accuracy, precision, linearity, robustness, and range. The validation findings suggest that these values are within the designated permissible ranges as specified by the ICH guidelines. A simple, cost-effective, specific, accurate HPLC technique has been developed and validated for the detection of Taraxerol. Reversed-phase chromatography was conducted using a C-18 column and a mobile phase consisting of Methanol + Water OPA (0.1%) (70:30% v/v) at a flow rate of 1.0mL/min. UV detection was conducted at a wavelength of 276 nm, resulting in a distinct peak for Taraxerol with a retention duration of 6.11 minutes. Maximum allowable %RSD for the peak region of the limit of quantification (LOQ) level is 2. The statistical analysis demonstrated that the approach was exact, accurate, selective, specific, and repeatable for the analysis of Taraxerol.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

## References

[1] Patwekar SL, Sakhare RS, Nalbalwar NN. HPLC method development and validation-A general Concept. International Journal of Chemical and Pharmaceutical Sciences. 2015;6(1):8-14.

[2] Ravisankar P, Navya CN, Pravallika D, Sri DN. A review on step-by-step analytical method validation. IOSR J Pharm. 2015 Oct;5(10):7-19.

[3] Bhagwat VR, Jain CM, Kochar NI, Sonar KS. Review on method development and validation for different pharmaceutical dosage form. GSC Biological and Pharmaceutical Sciences. 2024;26(3):055-67.

[4] Murugan S, Elayaraja A, Chandrakala K, Ramaiah P, Vulchi C. A review on method development and validation by using HPLC. International journal of novel trends in pharmaceutical sciences. 2013 Jul 10;3(4):78-81.

[5] Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An overview of experimental designs in HPLC method development and validation. Journal of pharmaceutical and biomedical analysis. 2018 Jan 5;147:590-611.

[6] Gupta V, Jain AD, Gill NS, Guptan K. Development and validation of HPLC method-a review. International research journal of pharmaceutical and applied sciences. 2012 Aug 31;2(4):17-25.

[7] Bhardwaj SK, Dwivedia K, Agarwala DD. A review: HPLC method development and validation. International Journal of Analytical and Bioanalytical Chemistry. 2015 Nov;5(4):76-81.

[8] Makasana J, Gajbhiye N, Kumar Bishoyi A, Savaliya M, Raju S, Bansod S, Baldaniya L, Dholakiya B. Exploration of HPTLC Technology for Rapid Chemical Fingerprinting and Simultaneous Determination of Bioactive Constituents from Clitoria ternatea Linn. ChemistrySelect. 2023 Apr 5;8(13):e202203217.

[9] Mus AA, Goh LP, Marbawi H, Gansau JA. The Biosynthesis and Medicinal Properties of Taraxerol. Biomedicines. 2022 Mar 30;10(4):807.

[10] Sharma K, Zafar R. Simultaneous estimation of taraxerol and taraxasterol in root callus cultures of Taraxacum officinale Weber. Int J Pharmacogn Phytochem Res. 2014;6(3):540-6.

[11] Jain PS, Chaudhari AJ, Patel SA, Patel ZN, Patel DT. Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. Pharmaceutical methods. 2011 Jul 1;2(3):198-202.