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AN ECO-FRIENDLY STABILITY-INDICATING RP-HPLC METHOD FOR THE QUANTITATIVE ANALYSIS OF ETRASIMOD IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, rapid, and green stability-indicating RP-HPLC method was developed and validated for the quantitative determination of etrasimod in bulk drug and pharmaceutical formulation. Chromatographic separation was achieved on a Prontosil ODS C18 column using an optimized mobile phase which is Ethanol and ammonium formate buffer in 65:35 (v/v) ratio at pH 4.6. A sharp and symmetrical peak of etrasimod was identified at a retention time of 2.49 min. The method demonstrated excellent specificity, with no interference from excipients or degradation products. System suitability parameters were observed to be within acceptable limits indicated by a tailing factor of 1.06 and 7,159 theoretical plates. LOD and LOQ values of 0.015 µg/mL and 0.05 µg/mL, exhibited high sensitivity. Linearity was developed in the concentration range of 5–30 µg/mL with a correlation coefficient (r^2) of 0.9994. % RSD values for precision were observed for intraday is 0.52%, 0.54% for interday and recovery was ranged from 98.76% to 100.63% according to the guidelines. Robustness and ruggedness studies indicated minimal variation under deliberate method changes. Forced degradation study confirmed the stability-indicating capability of the method, showing minor degradation under UV and thermal conditions, higher degradation under peroxide stress, and good stability under acidic and basic conditions. Application to the marketed Velsipity® formulation yielded an assay value of 99.05%. Greenness assessment using GAPI and AGREE tools confirmed reduced hazardous solvent usage without compromising analytical performance, supporting the method's suitability for routine quality control and stability studies.

INTRODUCTION:

An Active Pharmaceutical Ingredient (API) is the core component of a medicinal product responsible for producing an intended pharmacological response [1]. Accurate evaluation of the quality and concentration of APIs is essential in pharmaceutical analysis, as it directly influences drug safety and clinical efficacy. Among the analytical techniques employed for this purpose, reverse-phase high-performance liquid chromatography (RP-HPLC) is widely

recognized [2]. Owing to its high sensitivity, reproducibility, and versatility across a broad spectrum of pharmaceutical compounds, RP-HPLC has become an indispensable tool in both pharmaceutical research and routine quality assurance laboratories [3].Etrasimod is a novel, orally administered, selective sphingosine-1-phosphate (S1P) receptor modulator primarily targeting the $S1P_1$, $S1P_4$, and $S1P_5$ receptor subtypes [4]. It exerts its therapeutic effect by reducing the egress of lymphocytes from lymphoid tissues, thereby lowering circulating lymphocyte levels and modulating immune responses [5]. This mechanism makes etrasimod particularly effective in the management of immune-mediated inflammatory disorders, most notably moderately to severely active ulcerative colitis. Compared with earlier S1P receptor modulators. Etrasimod demonstrates improved receptor selectivity, which contributes to a favorable safety and tolerability profile, including a reduced impact on cardiac conduction [6]. Owing to its clinical significance and increasing therapeutic application, accurate and reliable analytical methods for the determination of etrasimod in bulk drug and pharmaceutical dosage forms are essential to ensure product quality, stability, and regulatory compliance [7-8].

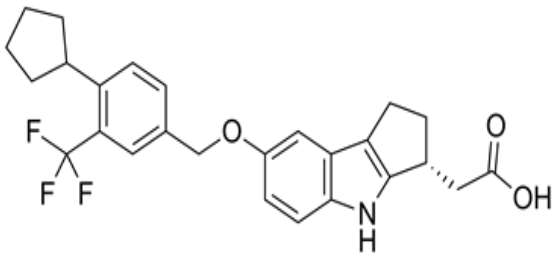


Figure 1: Etrasimod structure of the present study

Literature was studied in the view of analytical analysis for the estimation of etrasimod, only one HPLC/DAD/MS/MS analysis reported by Jelena et al., 2024 which focused on stability indication and greenness assessment [9]. However, a clear gap exists in the availability of a simple, validated RP-HPLC method suitable for routine estimation of etrasimod in bulk and dosage form samples. Addressing this gap is therefore necessary to support routine quality assessment and stability studies. Basing on these present objectives of the study are to develop a simple, rapid, and reliable RP-HPLC method for the quantitative estimation of etrasimod in bulk drug and pharmaceutical dosage forms.

Objectives of the study is

- To validate the developed method in accordance with ICH guidelines for parameters such as specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness.
- To evaluate the stability-indicating capability of the method by studying the degradation behavior of etrasimod under various stress conditions and assessing the separation of degradation products.
- To apply the validated method to the analysis of marketed pharmaceutical formulations of etrasimod.
- To assess the environmental friendliness (greenness) of the developed method using appropriate green analytical chemistry assessment tools.

METHODS AND MATERIALS

The etrasimod active ingredient (purity 98.94%) and the commercially available tablet formulation (Velsipity® 2 mg) were supplied by AR Life Sciences, Hyderabad. HPLC grade methanol, acetonitrile, and purified water were procured from Merck Chemicals Pvt. Ltd., Mumbai. Analytical reagent grade chemicals, including buffer solutions, hydrochloric acid, sodium hydroxide, and hydrogen peroxide used for the study, were obtained from Merck Specialties Pvt. Ltd., Mumbai, India.

Instrumentation:

Chromatographic analysis, the LC-7000 high-performance liquid chromatographic system consisting of an LC-P7000 solvent delivery pump (PEAK HPLC, India) was used. The sample injector system consists of a Rheodyne 7725 manual injector with a fixed sample loop volume of 20 µL, which used a Hamilton syringe (USA). UV detector was used to detect the analyte, Autochro-3000 software (Young Lin, Korea) was used. Equipment included a Denver SI-234 analytical balance (Bohemia), which was used for accurate weight, a Teccomp UV-2301 UV-Visible Spectrophotometer (India), which was utilized for preliminary analysis

or preliminary testing of the sample spectroscopically. A Systronics digital pH meter (Serial No. S-1326, India), which was utilized for pH adjustment or analysis of Buffer Solutions. Sample solutions were sonicated with the use of GT Sonic ultrasonic bath sonicator apparatuses (India). Mobile phase and sample solutions filtration of took place using borosilicate vacuum filtration apparatuses with 0.45 µm filters made from Merck Millipore, USA.

Preparation of standard solution:

A stock solution of etrasimod was made by weighing 10 mg of the compound and putting it in a 10 mL volumetric flask. The etrasimod was then dissolved in a methanol, the solution was brought up to the 10 mL mark with the same diluent, resulting in a final concentration of 1 mg/mL (1000 µg/mL). This stock solution was then diluted with the diluent to create working standards with concentrations from 40 to 140 µg/mL. Standard working solutions were kept at 4°C until they were analyzed.

Preparation of formulation solution:

A sample solution was created for the formulation analysis using commercially available Velsipity® tablets that were marked as containing 2 mg of etrasimod. A 10 mL volumetric flask with 5 mL of methanol was filled with precisely measured amounts of the powdered tablets, which equaled 10 mg of etrasimod. To fully extract the medication, the mixture was exposed to ultrasonic treatment for 5 mins. To remove insoluble excipients, the resultant solution was subsequently run through a 0.45 µm membrane filter. After using methanol to bring the volume up to the required level, the necessary subsequent dilutions were performed for formulation analysis.

HPLC Method Development:

The first step of method optimization involved selecting an appropriate wavelength for detection. This required a reference solution of 100 µg/mL of etrasimod to be prepared. This solution was scanned using a UV Visible spectrophotometer. This resulted in a scan giving details of its absorption. The wavelength representing maximum absorption of light was chosen. Etrasimod has a maximum absorption wavelength close to 249 nm. This wavelength has been used in all experiments. Since the compound to be analyzed was a polar compound, reversed-phase chromatography has been chosen, which required experimentation using C18 stationary phases. Columns of different sizes supplied by different companies are used.

To get the best result for the chromatographic system, various experimental conditions were varied. For the first experiment, the mobile phase was a mixture of Ethanol and water (75:25 v/v), and the system was set to a flow rate of 0.5 mL/min and a detection wavelength of 249 nm. It was carried out using the Kromosil ODS C18 Column (100 mm). For the second experiment, the eluent was varied and was now a mixture of Ethanol and 0.1% Formic acid in 80:20 v/v. However, the same parameters were maintained and were adjusted only by replacing the column with the 150 mm Prontosil ODS C18 column. For the third experiment, the mobile phase was now a mixture of Ethanol and 0.1% Formic acid (80:20 v/v), with the same detection parameters set at 249 nm and a flow rate set at 0.5 mL/min. This was carried out using the Prontosil ODS C18 column (150 x 4.6 mm and 3.5µm). For the fourth experiment, the composition was now changed to 20:80 v/v Ethanol and 0.1% Formic acid as a mobile phase. However, the same parameters were maintained. Refined experiments were carried out for experiments five and six using ternary solvent mixtures. For the fifth experiment, the eluent composition was 80:20 v/v Ethanol and ammonium formate buffer. For the sixth experiment, this was varied and was now 65:35 v/v with the same parameters maintained and done using the Waters Prontosil ODS C18 column.

HPLC Method validation:

The newly developed analytical method was evaluated for its performance characteristics such as linearity, accuracy, precision at intra and inter-day variations, specificity, and differentiation capability for the analyte based on the recommendations given regarding ICH guidelines for such analytical methods [10,11].

Specificity and System Suitability

Specificity was assessed for the

proposed analysis technique using various preparations such as blank, reference, and sample preparations for the formulation by performing the same chromatographic procedure for the different samples.The system suitability was established by performing six replicate injections of the standard solution. Important variables like system stability, symmetry, and column efficiency were taken into consideration.

Sensitivity

The sensitivity of the analytical procedure was evaluated for the determination of detection limit and quantification limit, which are the lowest concentrations that could be observed and determined.

Linearity and Range

The calibration profile was developed from the limit of quantification to a series of dilutions of a 5 mg/mL etrasimod working solution by an injection volume of 20 µL per analytical point and was repeated thrice to produce three analytical results at each dilution point.

Precision

The precision was determined based on an analysis of six replicates for the injection of etrasimod at a concentration of 20 µg/mL, using the percentage relative standard deviation. Repeatability, as well as intermediate precision, was carried out on the same day and over three different days, respectively.

% RSD = (standard deviation / mean) × 100.

Accuracy/Recovery

The accuracy of the methods was verified by recovery studies by using the standard addition method. Stock quantities of etrasimod were added to two different placebo preparations at three concentrations that were 50%, 100%, and 150% of the actual concentration. The percentage recovery was calculated based on the appropriate equation, and the result was expressed in terms of percentage recovery..

% RSD = (standard deviation / mean) × 100

Ruggedness

The ruggedness of the method was tested by assessing the impact of analyst variability. Two separate analysts separately prepared samples of concentration 80 µg/mL of etrasimod. The responses were compared, and the ruggedness of the method was determined based on %RSD values, taking <2% as the criterion.

Robustness

The robustness of the analytical method was explored by introducing small variations in the method parameters.

Formulation Analysis After validation, the developed method was used for determining etrasimod in commercial tablet dosage forms. Sample solutions were prepared at a strength of 80 µg/mL, and chromatographic separations were carried out under optimized conditions.

Forced degradation study:

The stability behaviors of etrasimod have been tested through comprehensive stress testing for 24 hours under various conditions of degradation [12, 13].

Acidic stress: A sample of 50 mg of the active ingredient was subjected to 50 mL of 0.1 N hydrochloric acid to bring about degradation by acid. After the degradation process, the sample was neutralized, diluted to a concentration of 80 °g/mL, and analyzed using the method.

Alkaline stress: For base hydrolysis, an equivalent amount of drug was treated with 50 mL of 0.1 N sodium hydroxide, and it was left for 24 hrs, followed by neutralization, dilution, and analysis.

Oxidative stress: For oxidative deterioration, 50 mg of the drug was used and it was subjected to oxidative stress by adding 50 mL of 3% hydrogen peroxide solution.

Photolytic stress: Photodegradation tests were carried out on a dose of 50 mg of the drug by exposure to ultraviolet radiation of wavelength 247 nm for a period of 24 hours. Thereafter, the sample preparation was done as in analytical procedures.

Thermal stress: Thermal stress was tested on the drug by taking 50 mg of the drug and submitting it to a temperature of 60 °C in a hot air oven for a period of 24 hours. Following the treatment, the test was analyzed.

RESULT AND DISCUSSIONS:

During method optimisation, a structured approach was adopted in which individual chromatographic parameters were

modified sequentially while all remaining conditions were held constant.The optimisation process primarily involved selecting an appropriate detection wavelength (Figure 2) using a UV–Visible spectrophotometer, along with careful selection of the stationary phase and mobile phase composition.

In the first experimental condition (Trial 1), the chromatographic output had a number of critical deficiencies, including poor separation, distorted peak geometry, unstable baselines, and the presence of extraneous peaks. The signals observed showed that the chromatographic configuration selected was incompatible for accurate and reproducible measurements. Incompatibility between the analyte, column surface, and mobile phase configuration selected was reflected by the unstable baselines and unknown signals. The chromatogram corresponding to this condition is presented in Figure 3A. To address these shortcomings, minor adjustments were introduced in the second experiment (Trial 2) with the intention of improving chromatographic stability and peak appearance. Despite these modifications, the resulting chromatogram displayed characteristics similar to those observed in Trial 1, such as persistent noise, asymmetric peaks, and unsatisfactory resolution. Consequently, further modifications to either the stationary phase or the eluent composition were deemed necessary. The chromatogram obtained under this condition is shown in Figure 3B. In Trial 3, the stationary phase was replaced with an alternative C18 column featuring different surface characteristics and column dimensions, with the objective of enhancing resolution and reproducibility. This change resulted in noticeable improvement in baseline smoothness and overall signal stability. Although interference was reduced and a single peak was obtained, the peak remained broad and system suitability criteria were not fully met. These findings demonstrated that while the revised stationary phase improved baseline behavior, additional optimization particularly of the mobile phase composition, buffer concentration, or pH was required to achieve a sharp and symmetrical chromatographic response. The chromatogram corresponding to Trial 3 is depicted in Figure 3C. In the fourth trial (Trial 4), the proportion of the aqueous component in the mobile phase was substantially increased in an attempt to enhance retention and peak definition. However, this adjustment failed to produce any meaningful improvement over Trial 3. The chromatogram continued to display a broadened peak with limited symmetry and no enhancement in separation efficiency. These results indicated that further refinement of the organic solvent proportion or inclusion of a buffering agent was required. The chromatogram for this condition is illustrated in Figure 3E. A substantial improvement was achieved in Trial 5 through the incorporation of ammonium formate buffer at pH 4.9 into the mobile phase. This resulted in a single, well-resolved, and symmetrical peak with minimal baseline noise. The enhanced resolution and system stability confirmed that the buffered mobile phase offered superior compatibility with ethanol as a green solvent. The chromatogram obtained under this condition is presented in Figure 3F. Building on the success of Trial 5, further refinement was carried out in Trial 6 by adjusting both the ratio of the organic phase and the buffer pH to optimise retention and peak symmetry. The use of ethanol and ammonium formate buffer in a 65:35 (v/v) ratio at pH 4.6 provided an optimal polarity balance, resulting in efficient elution and excellent peak characteristics. The chromatogram demonstrated a sharp, symmetrical peak with stable baseline and satisfactory system suitability parameters. Consequently, Trial 6 was selected as the final optimised green chromatographic condition for the quantification of etrasimod and was employed for subsequent method validation in accordance with ICH guidelines. The corresponding chromatogram is shown in Figure 3G.

Method Validation:

Specificity, and System Suitability

Method specificity was established through the assessment and comparison of chromatographic profiles derived from

reference, placebo, and formulation sample preparations. No interfering responses due to the tablet excipients were observed; the analyte eluted consistently at 2.49 minutes in all relevant chromatograms, while the blank exhibited no detectable signal at this retention region. These observations indicate that the method is capable of offering selective identification of etrasimod. The tailing factor for etrasimod under the optimized chromatographic conditions was 1.06, and the column efficiency corresponding to 7159 theoretical plates confirmed that system performance complied with the predefined acceptance criteria. Representative chromatograms are depicted in Figure 4.

Linearity and Range

For linearity, a series of combined standard solution concentrations of etrasimod spanning various levels, including the analytical concentration of 20 µg/mL, was prepared. A concentration of each was injected into the HPLC system twice using an injection volume of 20 µL. The mean peak areas were determined from the duplicate injections of each concentration, and the calibration curve was plotted using the average responses against the corresponding concentrations of each analyte. The analytical procedure was found to be very linear in the concentration range of 5 to 30 µg/mL. The linear regression equation was found to be $y = 11743x + 142473$, with a correlation coefficient (R^2) of 0.9994, establishing very linear responses over the tested concentrations. The linearity results are tabulated in Table 1, and the calibration plot is represented in Figure 5.

Precision and Ruggedness

Method precision was determined by the intra-day and inter-day variability studies and calculated using percentage relative standard deviation. Results from the repeatability test indicated a %RSD of 0.52%, while the intermediate precision from different days was estimated at 0.54%. Both values were below the acceptable limit of 2%, which reflected good consistency of the analytical procedure. Table 2. Results for intraday and interday precision. The ruggedness of the method was checked by analyzing the effect of analyst-to-analyst variability on the performance of analysis. Analysis was performed by two different analysts on different days using the same chromatographic system. The standard etrasimod solution (20 µg/mL) was analyzed in six replicates by both analysts. Ruggedness was considered acceptable according to %RSD, which was less than 2% of the calculated value.

Robustness

Robustness of the method was checked by introducing small deliberate variations in some important analytical parameters, such as detection wavelength, pH of the buffer, and mobile phase composition. Under each varied condition, a standard etrasimod solution at a concentration of 20 µg/mL was tested. The calculated % deviation lies between 0.22 and 1.35%, which falls within the pre-set limit of 2%. Data for robustness of the method are presented in Table 3.

Accuracy/ Recovery

The accuracy of the analytical method has been assessed by means of the standard addition method by spiking already analyzed sample solutions with appropriate amounts of etrasimod reference standard. The addition of spikes was done at three levels, viz., 50%, 100%, and 150% of the labeled claim. The %RSD achieved at these levels is 0.36%, 0.34%, and 0.65%, respectively. The recovery percentage is well within the acceptable range of 98–102%, and values of %RSD are well below 2%, establishing the accuracy of the developed analytical method. The recovery results for etrasimod are tabulated in Table 4, where values lie between 98.769 % and 100.631 %.

Formulation analysis The developed analytical method was validated, and it was further validated for estimation of etrasimod in a solution developed from the commercial preparation of Velsipity tablet formula. The result of the assay for etrasimod was found to be 99.05%, meaning a concentration of 19.8 µg/mL. The result confirms that the developed method is accurate for quality control analysis of etrasimod in bulk drugs and in pharmaceutical dosage forms.

Forced Degradation studies

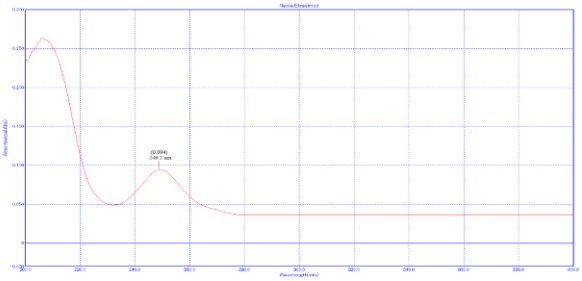
The HPLC analysis of etrasimod conducted under various stress conditions has shown the effective separation of the drug substance from its degradation product with no evidence of co-elution. The peak purity parameters satisfied the acceptable criteria for the stressed samples, ensuring the etrasimod peaks to be of high homogeneity. Forced degradation studies were also performed to evaluate the stability-indicating power of this established analytical tool. The tested samples underwent severe degradation under oxidation as well as basic conditions, resulting in two degradation products. Degradation was found to be 9.2% under peroxide conditions, with 90.8% of the original substance remaining. Degradation of 8.1% was noticed under basic conditions, with 91.9% of the original substance present. Hydrolysis showed moderate degradation of 6.3%, with a single degradation product, meaning that it is prone to hydrolysis.Very little degradation (5.9%) was seen due to the application of thermal stress at 60 °C; An assay of 97.1% was seen in the photolytic studies in addition to the degraded peaks seen above; this indicates that a high degree of stability is seen in etrasimod upon light stress. The results from the forced degradation studies verify the effectiveness of the HPLC assay in separating etrasimod from their degraded peaks. Selected chromatograms produced during the forced degradation experiments are shown in Figures 5.

Assessment of method greenness:

The AGREE evaluation of the proposed green RP-HPLC method yielded a score of 0.82, as shown in the pictogram. This high score indicates that the developed method is highly compliant with most of the GAC principles and demonstrates excellent environmental performance.The use of ethanol as the organic component in the mobile phase, in combination with ammonium formate buffer (pH 4.6), significantly contributed to the method's greenness by replacing toxic, non-biodegradable solvents such as acetonitrile and methanol. Ethanol is a renewable, biodegradable, and low-toxicity solvent, which reduced both chemical hazards and waste toxicity. The short run time (6 minutes) and low flow rate (0.5 mL/min) further enhanced the eco-efficiency of the method by minimizing solvent consumption and energy requirements.However, no severe environmental or safety hazards were identified, as the method avoids toxic, bioaccumulative, or persistent substances. Overall, the AGREE pictogram and score of 0.82 confirm that the developed RP-HPLC method for Etrasimod is environmentally sustainable, safe for operators, and analytically reliable. The results clearly demonstrate that the method successfully integrates green chemistry principles without compromising analytical performance, making it a highly suitable approach for routine quality control and stability testing of Etrasimod in both bulk and pharmaceutical formulations. The greenness assessment of the optimized RP-HPLC method for Etrasimod was further evaluated using the Green Analytical Procedure Index (GAPI) tool, which provides a visual representation of the environmental impact across all stages of the analytical workflow. The obtained GAPI pictogram (Figure 7) demonstrates the environmental profile of the method, using a three-color system where green denotes low environmental impact, yellow indicates moderate impact, and red represents high impact. Each pentagonal segment of the pictogram corresponds to a different stage of the analytical process — from sample collection and preparation to instrumental analysis and waste management. The GAPI pictogram of the developed method predominantly exhibited green-colored zones, indicating that the majority of the analytical steps are environmentally benign and well aligned with the principles of Green Analytical Chemistry. The central pentagon, which reflects the overall sustainability of the method, appeared yellow, representing a moderate environmental impact that is inherent to chromatographic techniques due to their solvent and energy requirements. However, the absence of any red zones confirms that no step in the procedure poses a significant environmental hazard. The sample preparation and reagent usage

sections were completely green, reflecting the method's reliance on ethanol and ammonium formate buffer, both of which are biodegradable and low in toxicity. No hazardous derivatization or extraction steps were involved, further supporting the eco-friendly nature of the procedure. The instrumental analysis section also displayed a strong green profile due to the method's short run time (5 minutes), low flow rate (0.5 mL/min), and the use of an energy-efficient isocratic elution mode, which collectively reduced solvent and power consumption. The waste generation and disposal section showed a mixture of green and yellow areas, indicating that although the total solvent waste was relatively low and composed of non-toxic materials, ethanol's flammability and the requirement for proper handling and disposal of buffered waste contribute a moderate environmental concern. The numerical value displayed (4.4E+02) represents the E-factor of 443, signifying that approximately 443 g of waste is produced per gram of analyte analysed. This value is substantially lower than typical HPLC methods using acetonitrile or methanol, which often exceed E-factors of 1000, demonstrating the superior environmental performance of the present method. Overall, the GAPI results confirm that the developed green RP-HPLC method for Etrasimod achieves an excellent balance between analytical efficiency and environmental sustainability. With predominantly green sectors, moderate central impact, and a comparatively low E-factor, the method can be considered sustainable, safe, and compliant with green chemistry principles, making it well-suited for routine quality control, formulation analysis, and stability testing with minimal ecological footprint.

Figure 2: UV scanning spectra of etrasimod



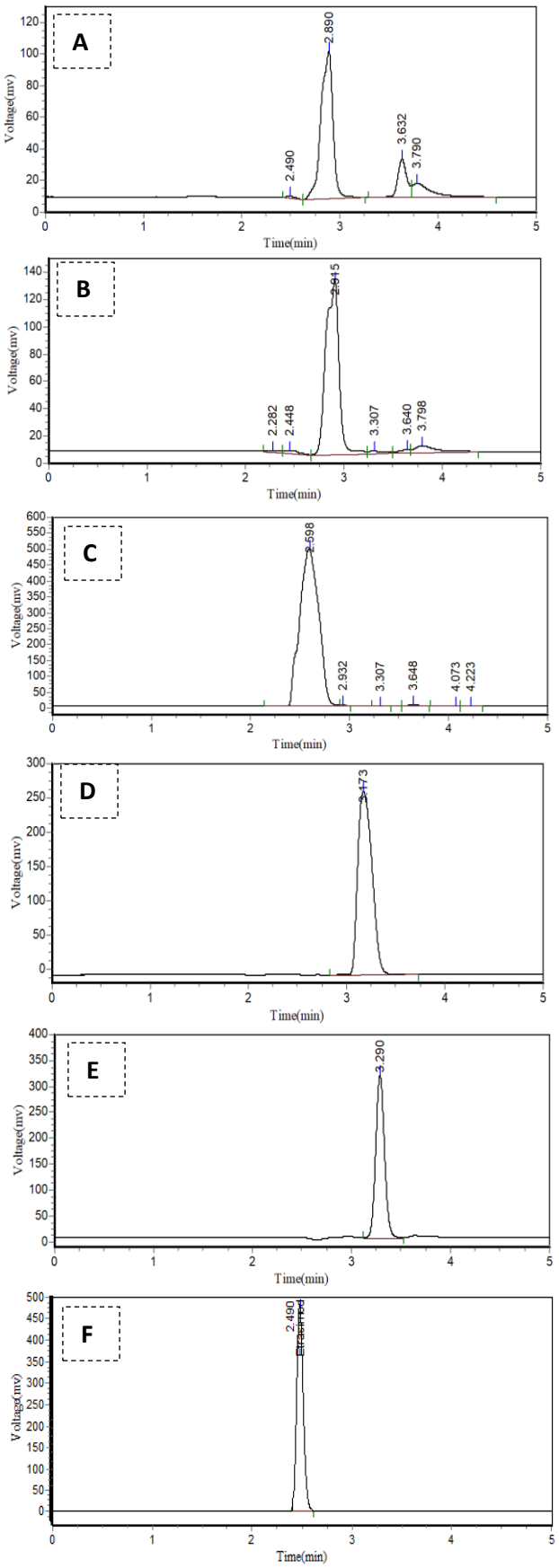


Fig3: Method development Chromatogram observed for etrasimod in (A) trail 1, (B) trail 2, (C) trail 3, (D) trail 4, (E) trail 5, and (F) trail 6 (or) optimized

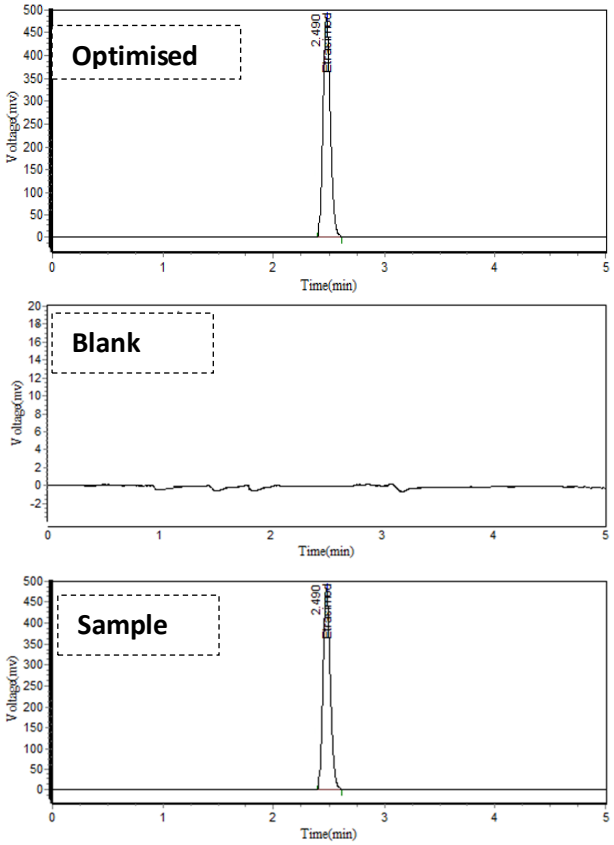


Fig4:Chromatogram observed for etrasimod

S.No	Concentration in µg/ml	Peak Area
1	5	203298.7
2	10	259872.6
3	15	317629.8
4	20	372505.3
5	25	438269.5
6	30	496297.1

Table 1: Linearity results for the etrasimod

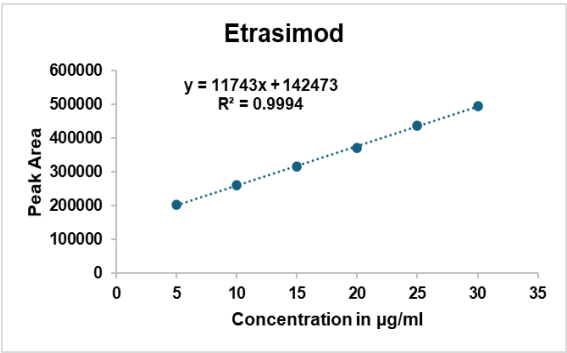


Fig 5: Linearity graph for the etrasimod

S. No	Intraday Precision	Interday Precision	Ruggedness
1	377538.9	375287.6	377467.2
2	376241.4	372654.3	378253.6
3	376387.6	377517.9	377837.1
4	377283.2	376524.8	377536.3
5	372175.8	377943.7	376745.5
6	375385.3	377629.1	377649.9
% RSD	**0.52**	**0.54**	**0.13**

Table 2: Precision and Ruggedness results for the etrasimod

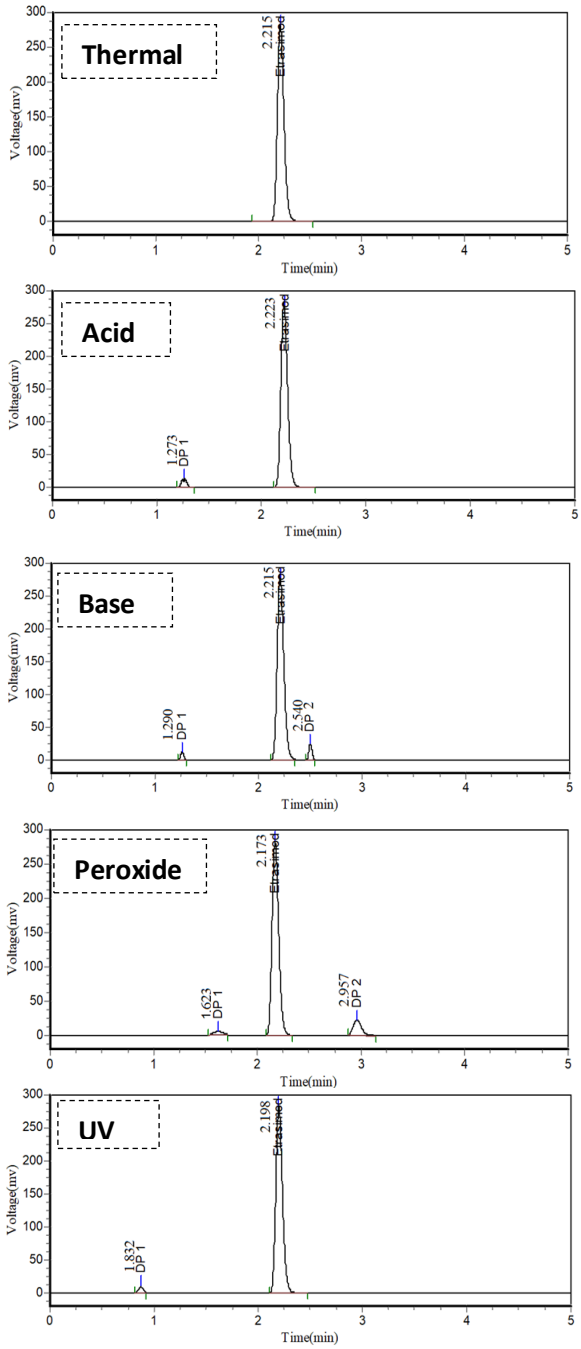


Figure 5: Chromatogram observed in UV Light degradation

S.No	Condition	Change	Area	% Change
1	Standard	No change	372505.3	---
2	MP 1	Ethanol: ammonium formate buffer at pH 4.6 in 60:40 (v/v)	371671.3	0.22
3	MP 2	Ethanol: ammonium formate buffer at pH 4.6 in 70:30 (v/v)	375624.5	0.84
4	WL 1	254 nm	373527.7	0.27
5	WL 2	244 nm	375547.4	0.82
6	pH 1	4.7	371583.6	0.25
7	pH 2	4.5	377546.2	1.35

Table 3: Robustness results for the etrasimod

% Recovery	Concentration in µg/ml			Amount Found	% Recovery	% RSD
	Target	Spiked	Total			
50%	10	5	15	15.011	100.075	0.36
	10	5	15	15.048	100.319	
	10	5	15	14.941	99.609	
100%	10	10	20	20.126	100.631	0.34
	10	10	20	20.114	100.569	
	10	10	20	20.002	100.012	
150%	10	15	25	24.976	99.905	0.65
	10	15	25	24.692	98.769	
	10	15	25	24.973	99.891	

Table 4: Accuracy results for the reported etrasimod

Fig 6: AGREE results observed for method proposed for the analysis of Etrasimod

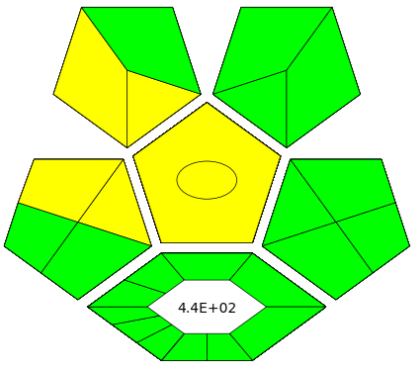


Figure 7: GAPI results observed for method proposed for the analysis of Etrasimod

CONCLUSION:

A sensitive reverse-phase HPLC method was developed and completely validated for the estimation of etrasimod in active pharmaceutical ingredient as well as in finished dosage forms. The chromatographic condition provided a well-resolved analyte signal at a retention time of 2.490 min, which was free from interference due to formulation components or degradation-related species. The chromatographic performance parameters studied ensured very good system performance as evidenced from the peak asymmetry value of 1.06 and column efficiency of 7159 theoretical plates. The proposed technique has high analytical sensitivity, as indicated by a very low detection limit of 0.015 µg/mL and quantitation limit of 0.05 µg/mL. The technique gave a very strong relationship between the concentration and the response for the concentration range of 5-30 µg/mL, yielding a correlation coefficient of 0.9994. The precision of the proposed technique was satisfactory, as evidenced by the low RSD%

of 0.52% for the repeatability study and 0.54% for the intermediate precision study. The accuracy of the proposed technique was confirmed by the recovery studies, which ranged between 98.769% and 100.631%. The ruggedness study using difference in operational conditions showed a low %RSD of 0.13% and a response variation of less than 2%. The stress study was a further confirmation that the proposed technique can distinguish etrasimod drug and its degrades. The quantitative analysis of the commercial velsipity® tablet resulted in an assay of 99.05%. The proposed technique meets the specifications of ICH Q2(R1) guidelines to be used for the routine analysis, contents, and stability study of etrasimod. In addition to the performance evaluation of the method, the environmental aspects of the approach were also investigated using the AGREE and GAPI evaluation methods. The evaluation of the approach using the AGREE method resulted in a greenness scale of 0.82. This indicated a high level of compatibility of the approach with the guidelines of Green Analytical Chemistry. The choice of ethanol as the organic modifier because of its renewable and biodegradable properties, in addition to the use of the ammonium acetate buffer system, ensured low environmental and toxicological hazards. Moreover, the shorter analysis time and lower flow rate of the mobile phase ensured a lower solvent consumption and waste production. The GAPI evaluation helped in confirming the eco-friendliness of the approach, with the majority of the segments in the green region of the cycle of the approach and the absence of the red region, ensuring the lack of environmentally critical steps in the approach. The E-factor value of 475 was further a measure of the tolerable waste production for the HPLC-based approach, especially taking into consideration the low toxicity and biodegradability of the solvents used in the approach. In conclusion, the new RP-HPLC method reported has been found to possess high selectivity, high sensitivity, high reproducibility, and quantitative ability for the estimation of etrasimod without the interference of the excipients and the degradation product. All validation criteria, including performance, calibration, robustness, and precision, have been found to have been validated according to the guidelines of the ICH Q2(R1) guidelines. The successful validation study on the estimation of velsipity® tablet samples and the method being stability-indicating make it suitable for use in laboratory testing and stability studies, and the method possesses potential and can be expanded for use in other dosage forms, biosamples, high-throughput analysis, and purification studies for regulatory purposes and pharmaceutical manufacturing needs, respectively.

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