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Development and Validation of a Stability-Indicating RP-HPLC Method for the Quantification of Repotrectinib in Drug Substance and Drug Production

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ABSTRACT

A straightforward, swift, and precise RP-HPLC method was devised and subsequently validated for the quantitative determination of repotrectinib in both its bulk form and in pharmaceutical preparations. Chromatographic separation was accomplished utilizing a C18 column and an optimised mobile phase, yielding a distinct, symmetrical peak corresponding to repotrectinib, which exhibited a retention time of 3.09 minutes. The method's specificity was found to be exceptional, with no observed interference from excipients or degradation products. Furthermore, system suitability parameters were maintained within acceptable ranges, as evidenced by a tailing factor of 1.05 and the presence of 4,891 theoretical plates, thereby signifying a high degree of column efficiency. The methodology exhibited considerable sensitivity, as evidenced by LOD and LOQ values of 0.09 µg/mL and 0.30 µg/mL, respectively, thereby facilitating the identification of minute quantities. A strong linear relationship was established throughout the concentration spectrum of 40–140 µg/mL (r^2 = 0.9998), thereby validating the method's exceptional linearity. Precision was confirmed through %RSD values of 1.20 (intra-day) and 1.22 (inter-day), while accuracy was substantiated by recovery rates spanning from 99.03% to 101.47%, all of which fell within the ICH guidelines. Furthermore, the method demonstrated both robustness and ruggedness, exhibiting minimal fluctuation in response to intentional alterations in analytical parameters. Forced degradation studies validated the method's capacity to indicate stability, revealing minor degradation under acidic (8.75%) and basic (6.32%) conditions, negligible degradation under oxidative stress (4.19%), and considerable stability under thermal (2.77%) and photolytic (1.99%) conditions. The absence of interference from degradation products further corroborated the assay's specificity and selectivity. When applied to the marketed Augtyro® (repotrectinib) formulation, the method produced an assay value of 99.47%, thereby demonstrating its appropriateness for routine quality control analysis. Consequently, this RP-HPLC method is characterized by its simplicity, sensitivity, precision, accuracy, robustness, and stability-indicating properties, rendering it highly suitable for the routine estimation and stability assessment of repotrectinib in both bulk and dosage forms.

Keywords: Repotrectinib, RP-HPLC, pharmaceutical, formulation, Forced degradation

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INTRODUCTION

An Active Pharmaceutical Ingredient (API) constitutes the principal element within a pharmaceutical formulation that elicits its therapeutic actions [1]. The precise assessment of API quality and quantity is of paramount importance in pharmaceutical analysis, serving as a critical determinant of drug safety and therapeutic effectiveness. reverse-phase high-performance liquid chromatographic (RP-HPLC) represents a frequently employed analytical methodology for this specific application [2]. RP-HPLC facilitates the meticulous separation, identification, and quantification of APIs within intricate mixtures through the passage of a liquid sample through a column containing a stationary phase. This technique is esteemed for its heightened sensitivity, accuracy, and capacity to accommodate a diverse array of pharmaceutical substances, thereby establishing its significance in both research endeavors and quality control laboratories [3].

Repotrectinib, marketed as Augtyro, is a next-generation tyrosine kinase inhibitor (TKI) specifically engineered to overcome resistance mechanisms in non-small cell lung cancer (NSCLC) associated with ROS1 gene mutations. ROS1 rearrangements represent a distinct oncogenic driver in NSCLC, and the solvent-front mutation ROS1 G2032R is particularly significant, accounting for 50–60% of cases that develop resistance to first-generation TKIs such as crizotinib [4]. The unique macrocyclic structure of repotrectinib confers several advantages: it minimizes interactions with common resistance mutation hotspots and is particularly effective against mutations occurring in the solvent-front region of the ROS1 kinase. This structural design allows repotrectinib to maintain efficacy where other TKIs—including crizotinib, lorlatinib, taletrectinib, and entrectinib—have shown limited success due to acquired resistance. Notably, to date, there have been no reported cases of resistance to repotrectinib, underscoring its potential as a durable therapeutic option [5].

On November 15, 2023, the FDA gave the green light to repotrectinib (Augtyro) for adults battling locally advanced or metastatic ROS1-positive NSCLC. This approval was buoyed by promising data from the TRIDENT-1 clinical trial. The trial showed an objective response rate (ORR) of 79% in patients who hadn't yet been treated with a TKI, and a 38% ORR in those who had. These figures underscore the considerable clinical advantage repotrectinib offers, whether used as a first-line treatment or when other TKIs have failed. In short, repotrectinib presents a new and effective treatment option for patients with ROS1-positive NSCLC, especially those who've developed resistance to current TKIs, marking a significant step forward in targeted cancer therapy [6]. Repotrectinib structure was given in Figure 1.

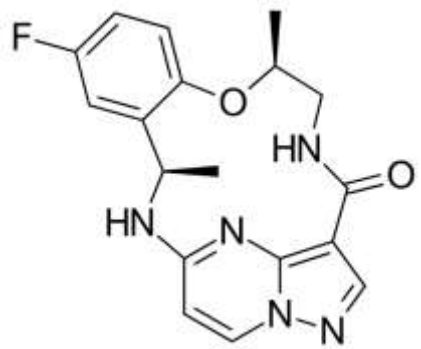


Figure 1: Repotrectinib structure of the present study

Repotrectinib molecular weight is 355.373 g/mol, molecular formula is $C_{18}H_{18}FN_5O_2$,

IUPAC name is *(3R,6S)-45-fluoro-3,6-dimethyl-5-oxa-2,8-diaza-1(5,3)-pyrazolo[1,5-a]pyrimidina-4(1,2)-benzenacyclononaphan-9-one*, Drug class is Tyrosine kinase inhibitor. Routes of administration is Oral. Literature was studied in the view of analytical analysis for the estimation of repotrectinib, single LC-MS/MS analysis reported by Siliveri A *et al.,* 2025 in plasma, and bioanalytical assay by using UPLC method by Wenlong Li et al., 2020 in mouse plasma [7, 8] are reported. There is a gap but there is gap for the estimation of RP-HPLC method for the estimation of repotrectinib in bulk and pharmaceutical formulation samples.

The primary aim of this investigation is to formulate and confirm an analytical procedure for quantifying repotrectinib in its individual dosage form, alongside an assessment of the degradation products [DPs] generated during the storage of the final pharmaceutical products. To accomplish these objectives, sophisticated methodologies, including RP-HPLC, will be utilized. The developed analytical method will be subjected to thorough validation, adhering to established regulatory standards. Essential validation parameters to be evaluated encompass accuracy, precision, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), selectivity, specificity, robustness, ruggedness, stability, and system suitability. The dependability and repeatability of the method across diverse circumstances hinge on each parameter's significance.

MATERIALS AND METHODS

The API of repotrectinib (99.52 % purity), and its tablet form (Augtyro® – 40 mg) were obtained from Bigbear Pharmaceutical as gift sample. Methanol, water, and acetonitrile, all of HPLC grade, were purchased from Merk Chemicals Pvt. Ltd., Mumbai. Furthermore, the analytical reagent (AR) grade buffer solutions, HCl, NaOH, and H_2O_2, employed in this study were sourced from Merck Specialties Pvt. Ltd., Mumbai, India.

Instrumentation

The analytical work was carried out using an LC-7000 HPLC system from PEAK HPLC, India, equipped with an LC-P7000 pump. Sample injections were performed manually using a Rheodyne type injector model is 7725 with a fixed volume loop of 20 µL, and a Hamilton (USA) syringe. Detection was achieved with a UV Detector, and data acquisition was managed by Autochro-3000 software from Young Lin (Korea). Additional equipment included a Denver SI-234 weighing balance (Bohemia), a Teccomp UV-2301 UV-Visible spectrophotometer (India), and a Systronics pH meter with serial number S 1326 (India). Sample preparation utilized a GT Sonic ultrasonic bath sonicator (India) and a borosilicate vacuum filtration kit. Filtration was performed using 0.45 µm membrane filters from Merck Millipore (USA).

Preparation of standard solution

A stock solution of repotrectinib was made by weighing 10 mg of the compound and putting it in a 10 mL volumetric flask. The repotrectinib 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 formulation solution was prepared for this study using Augtyro® tablets, each containing 40 mg of repotrectinib. First, a portion of the tablet powder, equivalent to 10 mg of repotrectinib, was carefully weighed and added to a 10 mL volumetric flask that already contained 5 mL of methanol. The flask's contents were then sonicated for 5 minutes to ensure the drug completely dissolved in the solvent. After this, the solution was filtered through a 0.45 µm membrane filter to remove any particles. The solution volume was then adjusted to the calibration mark of the flask using the same methanol as the diluent. Further dilutions were performed as needed during the formulation analysis.

HPLC Method Development

Method development process first step is to determine the optimal monitoring wavelength, standard solutions at a concentration of 100 µg/mL were prepared and subsequently analyzed using a UV-Vis spectrophotometer. The wavelength maxima identified were then chosen as the appropriate wavelength for detection. The UV absorption spectra of repotrectinib showed a maximum absorption at approximately 232 nm, which was therefore selected for use throughout the study. Given that repotrectinib is a polar compound, a non-polar C18 column was employed for the purpose of drug separation. Various columns, sourced from different manufacturers and featuring diverse configurations, were subjected to testing.

During the method development, we investigated several chromatographic parameters to improve the identification of the repotrectinib. In the first trial, the mobile phase was a 50:50 (v/v) mixture of methanol and water was utilized, detection occurred at 232 nm using a Zodiac C18 column (250 mm) and a flow rate of 1.0 mL/min. The second trial used a more organic solvent-rich mobile phase, specifically methanol and water in an 80:20 (v/v) ratio, while keeping the same wavelength and flow rate, but with a Hypersil GOLD™ C18 column (250 mm). In the third trial, a solvent mixture of acetonitrile and methanol (25:75, v/v) was used, maintaining the wavelength at 232 nm and a flow rate of 1.0 mL/min, and a Waters XBridge™ C18, 150 × 4.6 mm, 5 µm column was employed. Trail 4 used a mobile phase of acetonitrile and water in a 60:40 (v/v) ratio, while keeping the column, wavelength, and flow rate the same. Trails 5 and 6 then changed the mobile phase, using a three-component mixture: Trail 5 used methanol, acetonitrile, and water in a 70:20:10 (v/v) ratio, and Trail 6 adjusted this to 55:35:10 (v/v). Both trails used the Waters XBridge™ C18 column, with the same wavelength and flow rate. These changes were made to find the best chromatographic conditions for the analysis.

HPLC Method validation

The analytical method's performance was validated, encompassing linearity, trueness, reproducibility, repeatability, discrimination, and selectivity, in accordance with ICH guidelines [9-11].

Specificity and System Suitability

To assess specificity, the method was challenged by introducing diverse solutions specifically, reference, blank, and formulation samples into the chromatograph. Chromatograms of repotrectinib derived from both standard and sample preparations were compared with those obtained from tablet samples to ascertain the method's capacity to differentiate the analyte. Furthermore, retention times were compared to confirm the method's capability to accurately identify repotrectinib.

System suitability was assessed by performing six consecutive injections of the reference solution, examining criteria such as peak area, USP tailing factor, and theoretical plate count for repotrectinib. Acceptance parameters included a % RSD not exceeding 2%, resolution of at least 2, a USP tailing factor not greater than 2, and a theoretical plate count of at least 2000.

Sensitivity

Sensitivity was determined by establishing the LOD and LOQ, defined as the smallest detectable and quantifiable analyte concentrations. Standard solutions were diluted accordingly, and LOD and LOQ were established based on signal-to-noise ratios of 3 and 10, respectively, for each compound by analyzing progressively diluted samples.

Linearity and Range

A calibration curve was generated starting from the LOQ concentration, using a repotrectinib stock solution (1 mg/mL) and preparing serial dilutions. Each concentration was analyzed with a 20 µL injection, and all measurements were performed in triplicate. The correlation between peak area and concentration was assessed through graphical representation, and correlation coefficients and regression parameters were subsequently determined.

Precision

Precision was gauged by subjecting repotrectinib to six analyses at an 80 µg/mL concentration, with the outcomes presented as % RSD. Both repeatability (intra-day) and intermediate precision (inter-day, spanning three days) were evaluated, and % RSD was computed employing the formula:

$$\% \text{ RSD} = (\text{standard deviation} / \text{mean}) \times 100.$$

Accuracy/Recovery

Accuracy was ascertained via recovery experiments, wherein two placebo solutions were spiked with known quantities of repotrectinib at three distinct concentration levels (50 %, 100 %, and 150 %).

The standard addition method was employed to determine the percentage of drug recovered, utilizing the pertinent formula.

$$\% \text{ RSD} = (\text{standard deviation} / \text{mean}) \times 100$$

Ruggedness

Ruggedness was evaluated by having two analysts independently prepare repotrectinib solutions at a concentration of 80 µg/mL, maintaining consistent laboratory conditions. The peak areas obtained were then assessed, and the method's ruggedness was expressed as % RSD, with a threshold of less than 2% mandated.

Robustness

To ascertain robustness, the method underwent six deliberate modifications, including variations in the mobile phase ratio and detector wavelength, each within ± 5 % of the nominal conditions. The effect of each modification was quantified and presented as a % change.

Formulation Analysis

The validated method was subsequently employed to quantify repotrectinib in commercially available tablet formulations. Samples at a concentration of 80 µg/mL were analyzed under the established conditions, and the peak area was utilized to calculate the assay percentage, employing the label claim recovery approach and the calibration curve for this purpose.

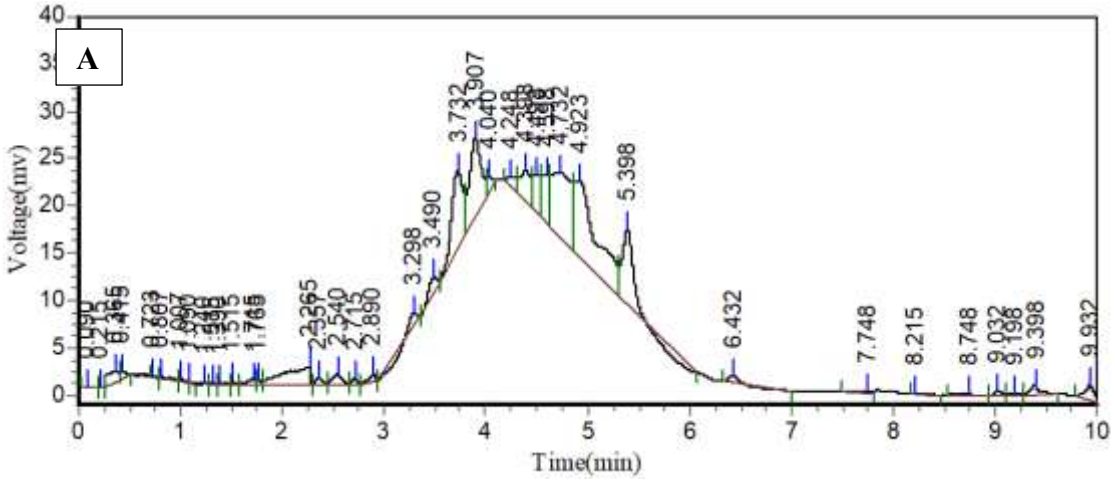
Forced degradation study

To thoroughly evaluate the drug stability, a series of forced degradation studies were performed, subjecting the drugs to various stress conditions for a duration of 24 hours. Acid hydrolysis assessment, 50 mg of the drug was meticulously combined with 50 mL of a 0.1N HCl solution, thereby simulating acidic environments. Following the stress exposure, the solution underwent neutralization, and then diluted to a standard concentration of 80 µg/mL, and was then analyzed employing the established analytical method. Similarly, for the base hydrolysis evaluation, the same quantity of drug was combined with 50 mL of a 0.1N NaOH solution to replicate basic conditions, 24 hours interval, neutralization and dilution procedures were followed prior to analysis. To induce oxidative degradation, 50 mg of the drug underwent treatment with 50 mL of a 3% hydrogen peroxide solution, thereby simulating oxidative stress. Subsequent to this treatment, the solution was neutralized and reconstituted to the standard concentration before analysis. Photolytic degradation study was employed to the exposure of 50 mg of the drug sample to UV light at a wavelength of 247 nm for a duration of 24 hours, thereby simulating the effects of light exposure. Subsequent to this phase, the drug solution underwent preparation for analytical assessment, adhering to established protocols. Ultimately, thermal degradation was assessed by subjecting a 50 mg drug sample to an oven maintained at 60°C for a duration of 24 hours, thereby simulating the effects of elevated temperatures. Following this thermal exposure, the sample was processed and analyzed utilizing the previously established methodology. These methodical investigations facilitate a comprehensive understanding of the drug's stability characteristics when exposed to diverse environmental and chemical stressors, thereby ensuring the drug's reliability and efficacy throughout its designated shelf life [12].

RESULT AND DISCUSSIONS

In method development process the initial chromatographic run yielded a chromatogram of inadequate resolution; instead of a singular, symmetrical peak representing repotrectinib, the data presented broad, indistinct signals across various retention times, coupled with a highly unstable baseline. This observation implies co-elution and an inconsistent detector response. Consequently, this result suggests that the initial configuration, encompassing the stationary phase, mobile phase composition, and detection wavelength, lacked sufficient selectivity and elution power to effectively separate the analyte from the matrix and associated compounds, likely exacerbating baseline noise. Trial 1 (Figure 2 A), in contrast to the ideal chromatograms described in the literature and those achieved during later optimization, showed a single, sharp peak and a stable, flat baseline. This result highlighted the need to change the stationary phase and to re-evaluate both the detection wavelength and the sample solvent. The

goal was to improve peak shape, retention consistency, and baseline stability. In the second trail [Figure 2B], the addition of methanol to the mobile phase, alongside the employment of a Hypersil GOLD™ C18 column, resulted in a single, dominant peak for repotrectinib. Nevertheless, this peak displayed a broad profile, an unstable baseline, and irregular retention properties. Although this method improved elution and reduced the unresolved peaks seen in Trial 1, the system's selectivity and stability were still inadequate. These observed shortcomings suggested possible explanations, including incomplete solubility, column overloading, or an incompatibility between the mobile phase and the analyte. Therefore, while this trial showed some progress compared to Trial 1, it ultimately did not achieve sufficient resolution or baseline stability, thus requiring further optimization. In the third trial [Figure 2C], replacing water with a mixed organic system of acetonitrile and methanol and using a shorter Waters XBridge™ C18 column significantly improved chromatographic behavior, producing a single, well-detected peak with a stable baseline. This outcome confirmed better column compatibility and overall system stability compared to the previous two trials. However, despite the improved baseline and reproducible retention, the observed peak exhibited pronounced tailing and inadequate resolution, indicating incomplete optimization of the mobile phase polarity or lack of a suitable aqueous component or modifier. Compared with Trial 2, this trial marked a substantial improvement in baseline stability and peak consistency but revealed the necessity of fine-tuning the mobile phase composition to achieve sharper peak symmetry and improved separation efficiency. In the fourth experiment, methanol was substituted with water in the mobile phase, while the acetonitrile ratio and chromatographic conditions remained constant. The outcomes were similar to those observed in Trial 3, producing a single, broad peak with considerable tailing and no enhancement in peak sharpness or resolution. The consistent baseline and inadequate separation suggested that altering the organic component alone, without incorporating a pH modifier or buffer, did not effectively improve peak symmetry (Figure 2D). This trial further validated that acetonitrile–water mixtures alone were insufficient for resolving or sharpening the repotrectinib peak, thereby underscoring the necessity of acidic modifiers or gradient elution to optimize chromatographic performance. In the fifth trial, the employment of a ternary solvent system comprising methanol, acetonitrile, and water (70:20:10, v/v) yielded a stable baseline characterized by minimal noise and a singular, well-defined peak corresponding to repotrectinib. This outcome suggested superior solvent compatibility and detector stability relative to earlier trials. Nevertheless, the peak exhibited a degree of broadening, and system suitability parameters, including the tailing factor and theoretical plates, remained below the established acceptable thresholds (Figure 2E). Although this methodology enhanced baseline clarity and peak consistency in comparison to Trial 4, chromatographic efficiency was still inadequate, thereby underscoring the necessity for further optimization to attain acceptable system suitability. In the sixth trail, the mobile phase was modified to a methanol, acetonitrile, and water mixture (55:35:10, v/v), which led to enhanced chromatographic behavior. This adjustment produced a well-defined, symmetrical peak characterized by superior baseline stability and a consistent retention time. This specific composition mitigated the peak broadening and tailing observed in earlier trials, while simultaneously preserving a dependable detector response and acceptable system suitability. Relative to Trial 5, the augmented acetonitrile concentration enhanced elution strength and selectivity, thereby improving resolution and overall efficiency, as illustrated in Figure 2F. Consequently, these conditions were considered appropriate for subsequent assessment.



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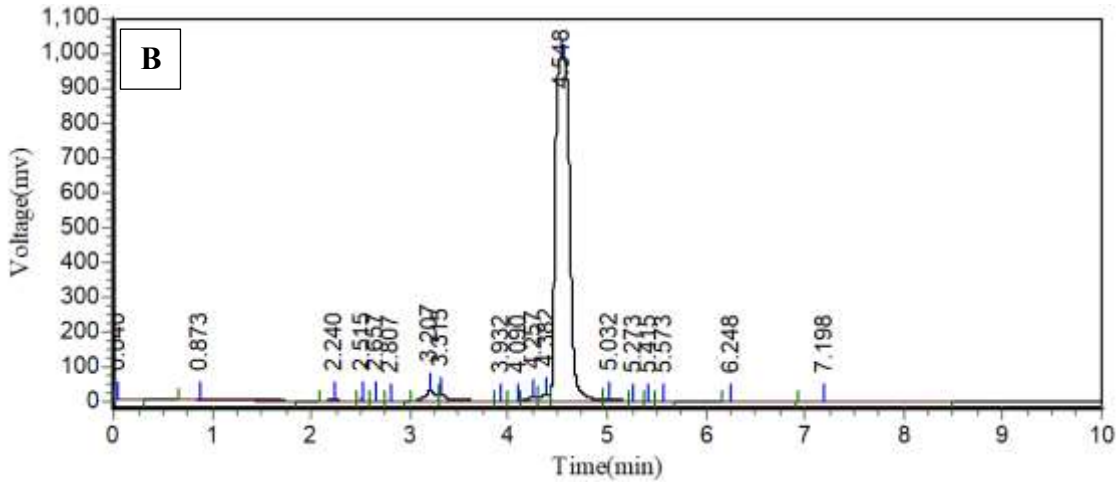

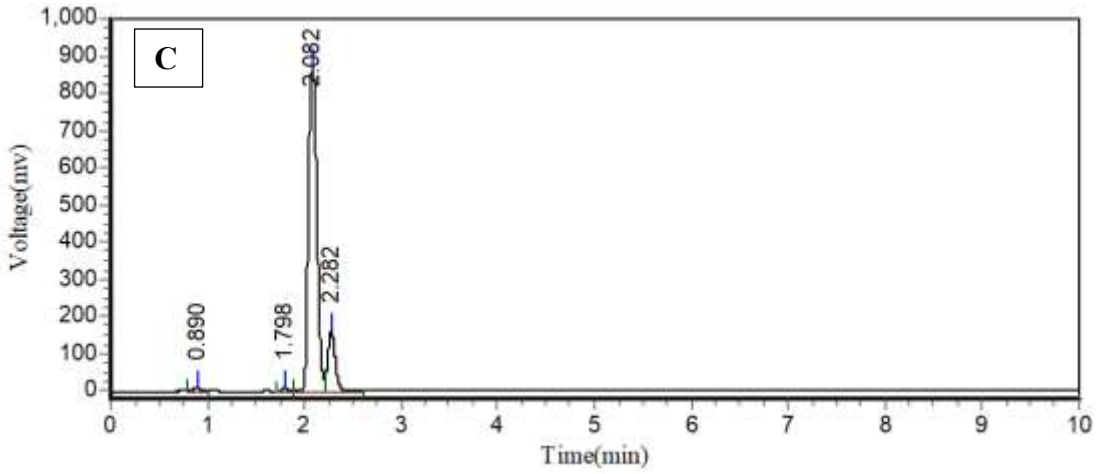

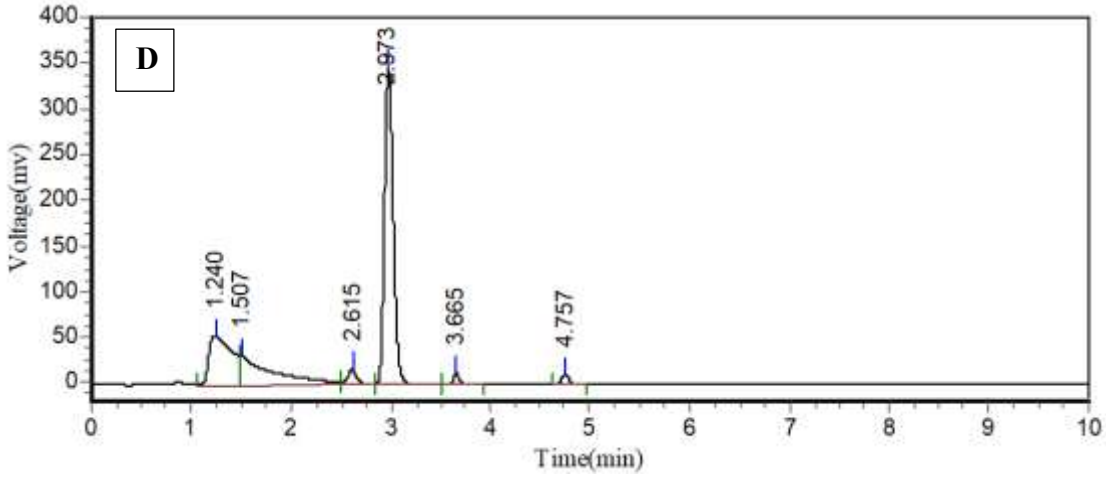

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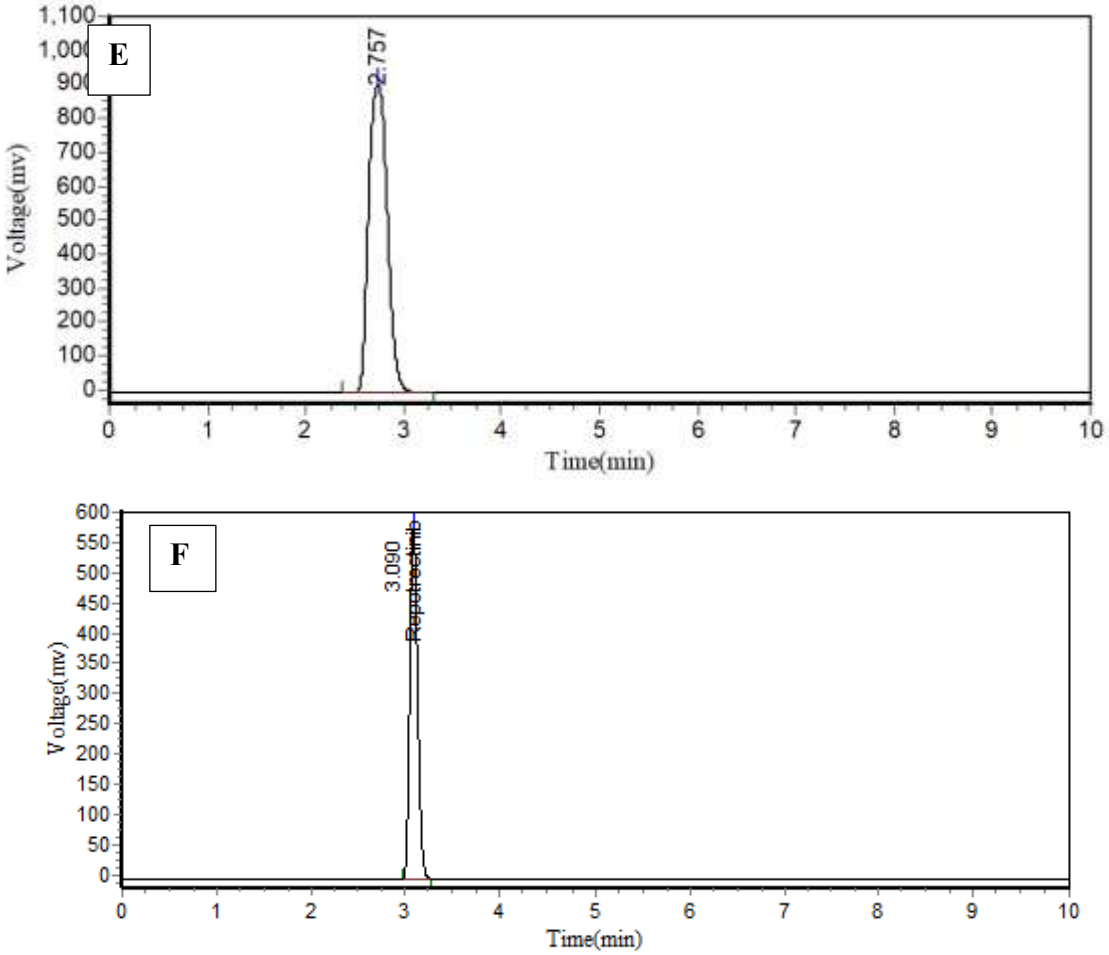

Figure 2: Method development Chromatogram observed for repotrectinib in (A) trail 1, (B) trail 2, (C) trail 3, (D) trail 4, (E) trail 5, and (F) trail 6 (or) optimized

Method Validation:

Specificity, and System Suitability

The method's specificity was established through a comparative analysis of chromatograms derived from standard, blank, and sample solutions. The absence of interference from tablet excipients was noted, and the retention times for repotrectinib remained uniform across all solutions (3.09 min), with no discernible peak in the blank. Consequently, these findings validate the method's specificity and selectivity.

Under the optimized conditions, the peak tailing factor for repotrectinib was 1.05, and the number of theoretical plates was 4,891. These results indicate that the method meets the established requirements. Chromatograms are given in Figure 3.

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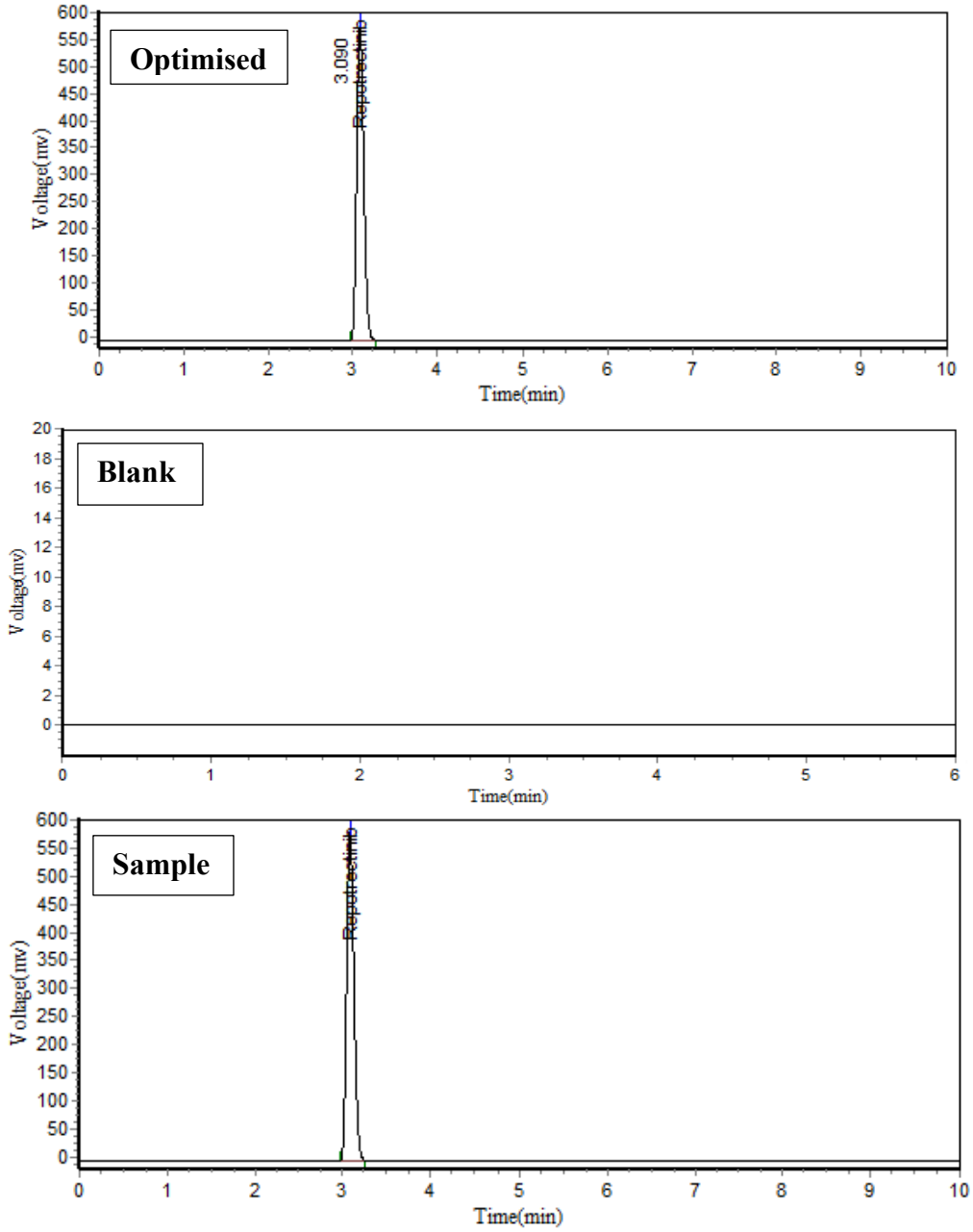

Figure 3: Chromatogram observed for repotrectinib

Sensitivity

The LOD and LOQ for repotrectinib were determined to be 0.09 µg/mL and 0.300 µg/mL, respectively. These results confirm that the method is highly sensitive and suitable for detecting and analyzing the drug at very low concentrations.

Linearity and Range

Linearity was evaluated through the preparation of a series of mixed standard solutions of repotrectinib, encompassing a range of concentration levels, including the working concentration defined by the experimental parameters (80 µg/mL). Each concentration was subjected to duplicate injections into the HPLC system, with a sample injection volume of 20 microliters. The detector response was monitored at a wavelength of 232 nm, and the resultant chromatograms were meticulously documented.

The average peak areas for each concentration were determined from the duplicate injections, and calibration plots were constructed by graphing the mean peak area against the corresponding repotrectinib concentrations. This method exhibited exceptional linearity across the concentration spectrum of 40–140 µg/mL. The linear regression equation derived from the calibration curve was y = 27747x + 65081, with a correlation coefficient (R^2) of 0.9998, thereby signifying a highly linear response within the evaluated range. Linearity results are given in Table 1, and calibration curve was given in Figure 4.

Table (1): Linearity results for the repotrectinib

S. No	Concentration in µg/ml	Peak Area
1	40	1165478.5
2	60	1745896.6
3	80	2269783.9
4	100	2855974.2
5	120	3391006.6
6	140	3945764.4

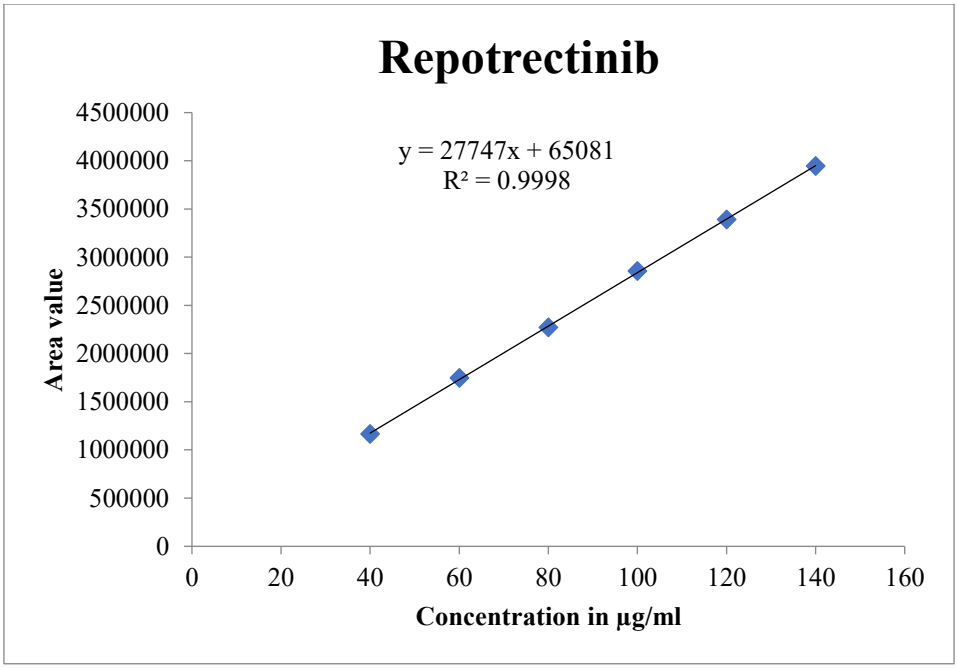

Figure 4: Linearity graph for the repotrectinib

Precision and Ruggedness

The intraday precision of repotrectinib, as assessed by the % RSD, was found to be 1.20 %, while the interday precision was 1.22 %. These values are both below the established threshold of 2%, thereby suggesting that the method exhibits a high degree of precision. In accordance with this premise, the analytical method developed is deemed precise for the quantification of repotrectinib. Intraday and Interday Precision results are given in Table 2.

Ruggedness, on the other hand, is assessed by having different analysts perform the analysis on different days. In this study, ruggedness was evaluated by changing the analyst while using the same analytical instrument. The developed method was employed to analyze a standard solution at a concentration of 80 µg/mL, with two analysts conducting six replicate analyses for each analyte. Ruggedness was evaluated through the calculation of the percentage relative standard deviation (% RSD),

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where an acceptable value is defined as less than 2%. The % RSD determined in this investigation was 1.17, thereby demonstrating the method's ruggedness and confirming that the results (Table 2) are within the established acceptance criteria.

Table 2: Precision and Ruggedness results for the repotrectinib

S. No	Intraday Precision	Interday Precision	Ruggedness
1	2269783.6	2269718.2	2249873.8
2	2254789.3	2287964.8	2291486.7
3	2214965.7	2219738.4	2298476.3
4	2235794.2	2279843.1	2298748.9
5	2285349.8	2278964.9	2241963.5
6	2279139.4	2298746.3	2298716.2
% RSD	**1.20**	**1.22**	**1.17**

Robustness

A robustness assessment was performed by implementing minor adjustments to the wavelength, buffer pH, and mobile phase composition. Repotrectinib, at a concentration of 80 µg/mL, was subjected to analysis under these modified experimental parameters. The observed percentage change for repotrectinib spanned from 0.42 % to 1.27 %, remaining within the acceptable threshold of less than 2 %. Consequently, these findings validate those subtle alterations in analytical conditions do not substantially impact the results, thereby indicating that the proposed method is robust and appropriate for repotrectinib analysis, even with slight variations in experimental parameters. Robustness results are given in Table 3.

Table 3: Robustness results for the repotrectinib

S. No	Condition	Change	Area	% Change
1	Standard	No change	2269783.9	0.00
2	MP 1	Methanol: Acetonitrile: Water 50:40:10 (v/v)	2247863.4	0.97
3	MP 2	Methanol: Acetonitrile: Water 60:30:10 (v/v)	2298719.2	1.27
4	WL 1	227 nm	2249173.5	0.91
5	WL 2	237 nm	2281379.6	0.51
6	pH 1	5.9	2279378.7	0.42
7	pH 2	5.7	2243981.8	1.14

Accuracy/ Recovery

In this study, we added known amounts of a standard solution to the previously analyzed sample solutions. These additions were made at levels corresponding to 50 %, 100 %, and 150 % of the stated claim. The RSD values for repotrectinib, measured at the 50 %, 100 %, and 150% spiked levels, were 1.24 %, 0.87 %, and 0.44 %, respectively. The results, which showed recoveries between 98% and 102% and RSD of less than 2%, confirmed the accuracy of the proposed method. Table 4 shows the recovery results for repotrectinib, with the recovery percentages ranging from 99.03 % to 101.47 %.

Table 4: Accuracy results for the reported repotrectinib

% Recovery	Concentration in µg/ml			Amount Found	% Recovery	% RSD
	Target	Spiked	Total			
50%	40	20	60	60.412	100.69	1.24
	40	20	60	60.881	101.47	
	40	20	60	59.418	99.03	
	40	40	80	80.879	101.10	0.87

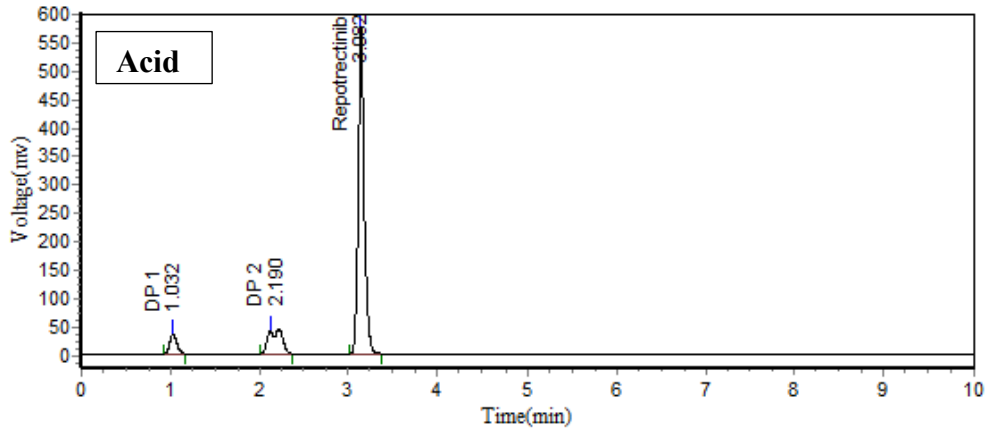
100%	40	40	80	79.611	99.51	
	40	40	80	80.763	100.95	
150%	40	60	100	101.357	101.36	0.44
	40	60	100	100.469	100.47	
	40	60	100	100.834	100.83	

Formulation analysis

The method was used to measure the amount of Repotrectinib in a solution made from the Augtyro® pharmaceutical formulation. Using this method, the assay percentage for Repotrectinib was found to be 99.47% (79.58 µg/mL). The method consistently produced assay results exceeding 98%. Therefore, this method is useful for regularly checking the quality of Repotrectinib, both in its raw form and in the final pharmaceutical products.

Forced Degradation studies

Repotrectinib's HPLC analysis revealed no interaction between the drug and its degradation products when exposed to various stress conditions. The peak purity data remained within acceptable parameters across all degradation scenarios, thereby validating the homogeneity of the repotrectinib peak. This finding confirms the assay method's specificity and its capacity to indicate stability. Repotrectinib was successfully separated, identified, and quantified under all stress degradation conditions, encompassing acidic, basic, oxidative (peroxide), thermal, and UV light exposure. The forced degradation study was performed to evaluate the stability-indicating properties of the developed HPLC method. Notably, significant degradation was observed during acidic hydrolysis, yielding two distinct degradation products and a total degradation of 8.75 %, while 91.25 % of the parent compound remained unaltered. Alkaline hydrolysis yielded a single degradation peak, corresponding to a 6.32 % degradation rate, thereby suggesting a moderate vulnerability to basic environments. Conversely, oxidative stress induced by hydrogen peroxide led to negligible degradation (4.19 %), and no supplementary peaks were detected, which illustrates considerable stability under oxidative conditions. Likewise, exposure to thermal and photolytic (UV light) stress did not generate any novel degradation peaks; the remaining assay values were 97.23% and 98.01%, respectively, thus indicating that repotrectinib exhibits substantial stability to both heat and light. The findings collectively validate the efficacy of the developed method in effectively isolating repotrectinib and its degradation products when subjected to stress conditions. This establishes the method as a specific, dependable, and stability-indicating HPLC technique, thereby rendering it appropriate for both routine quality control and stability assessments. The comprehensive results derived from the forced degradation study associated chromatograms are illustrated in Figures 5.



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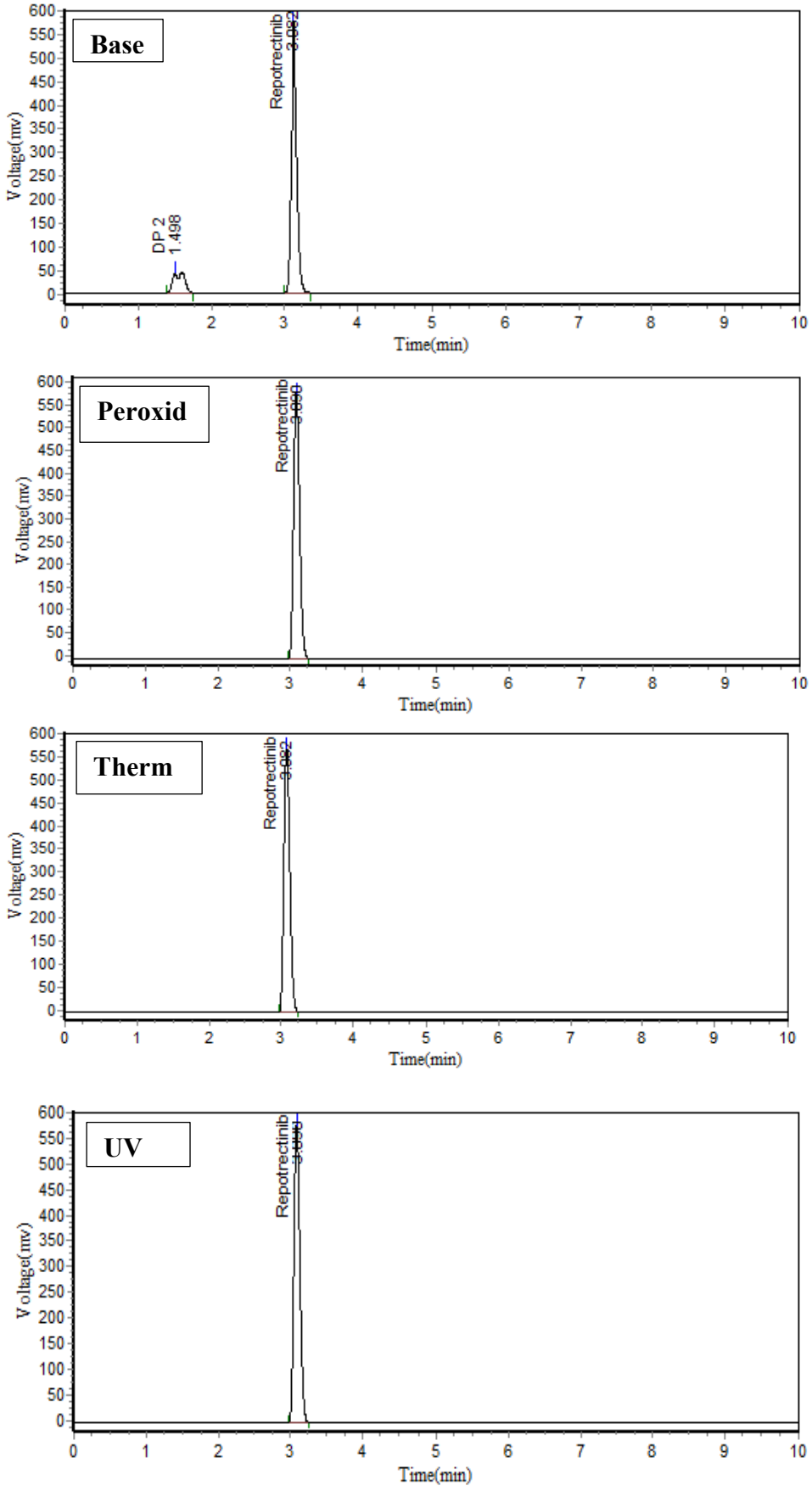

Figure 5: Chromatogram observed in UV Light degradation

CONCLUSION

A straightforward, accurate, and stability-indicating RP-HPLC method was devised and subsequently validated for the quantification of repotrectinib in both its bulk form and within pharmaceutical preparations. The method produced a distinct peak at 3.09 min, with no observed interference from excipients or degradation products. System suitability assessments Validated the method's superior performance, as evidenced by a tailing factor of 1.05 and 4891 theoretical plates. Furthermore, the method exhibited considerable sensitivity (LOD: 0.09 µg/mL; LOQ: 0.30 µg/mL), robust linearity (40–140 µg/mL, R^2 = 0.9998), and high precision, as indicated by % RSD values of 1.20 for intra-day and 1.22 for inter-day measurements. The method's accuracy ranged from 99.03 % to 101.47 %, and the ruggedness and robustness were confirmed by % RSD and % change values below 2 %. Forced degradation studies confirmed the method's ability to indicate stability. The assay of Augtyro® tablet was found to be 99.47 %. Therefore, this RP-HPLC method meets the ICH Q2(R1) guidelines, making it suitable for routine quality control, assay, and stability testing of repotrectinib.

In summary, the developed RP-HPLC method for the quantification of repotrectinib demonstrates excellent specificity, sensitivity, precision, and accuracy, with no interference from excipients or degradation products. System suitability, linearity, and robustness were thoroughly validated in accordance with ICH Q2(R1) guidelines. The method reliably quantifies repotrectinib in both bulk and pharmaceutical formulations, as evidenced by its successful application to Augtyro® tablets. Its stability-indicating capability further enhances its utility for routine quality control, assay, and stability testing of repotrectinib in pharmaceutical settings. This validated RP-HPLC method can be further explored for application in analyzing repotrectinib in other pharmaceutical dosage forms and complex biological samples. Additionally, its adaptation for high-throughput and impurity profiling could support broader quality control and regulatory requirements in pharmaceutical research and manufacturing.

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