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Analytical Method Development and Validation for Quantitative Estimation of Tebentafusp Bulk Dosage by RP-HPLC

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Abstract: *This study evaluates the analytical method for Tebentafusp (TEBN) in Kimmtrak Injection using HPLC with a photodiode array detector. The mobile phase was prepared with a 50% mixture of KH_2PO_4 buffer and methanol, sonicated, and filtered. Standard and sample solutions of TEBN were prepared, and various trials were conducted to optimize the chromatographic conditions. Stability tests were performed under different conditions including 0.1N HCl, 0.1N NaOH, 30% peroxide, sunlight, and 105°C. Trials revealed issues with peak shape and resolution, leading to multiple adjustments in mobile phase composition and column selection. The final optimal conditions were achieved with a KH_2PO_4: Methanol (60:40) mobile phase on a Thermo C18 column at a flow rate of 0.8 ml/min, with acceptable peak shape and system suitability parameters. Validation according to ICH guidelines demonstrated linearity, precision, accuracy, and selectivity. Linearity was confirmed within the range of 25-75 µg/ml with a detection limit of 0.076 µg/ml and quantitation limit of 0.255 µg/ml. Precision was indicated by a low relative standard deviation, and accuracy was confirmed by recovery studies. The method proved robust and suitable for the quantification of TEBN in Kimmtrak Injection, ensuring reliable quality control in pharmaceutical analysis.*

Keywords: Tebentafusp, HPLC, photodiode array detector, Kimmtrak Injection, chromatographic optimization, stability tests, method validation, pharmaceutical analysis

I. INTRODUCTION

The quality assurance of pharmaceutical products is a critical aspect of drug development and production, ensuring that medications are safe, effective, and of consistent quality. [1,2]Tebentafusp, an innovative bispecific protein for the treatment of metastatic uveal melanoma, represents a significant advancement in oncology therapeutics. Accurate and reliable analytical methods are essential to quantify Tebentafusp in its dosage form, particularly in Kimmtrak Injection, to ensure its therapeutic efficacy and safety.[3,4]

High-Performance Liquid Chromatography (HPLC) is a widely accepted analytical technique for the separation, identification, and quantification of components in pharmaceutical formulations.[5] The precision, accuracy, and reproducibility of HPLC make it an indispensable tool in quality control laboratories. However, the development and validation of an HPLC method tailored specifically for Tebentafusp in Kimmtrak Injection presents unique challenges, given the complex nature of the bispecific protein and the need for stringent regulatory compliance.[6]

This research aims to develop and validate a robust HPLC method using a photodiode array detector (PDA) for the quantification of Tebentafusp in Kimmtrak Injection. The method development process involves optimizing chromatographic conditions to achieve adequate resolution, sensitivity, and specificity. Subsequently, the method will undergo rigorous validation according to International Conference on Harmonisation (ICH) guidelines, assessing parameters such as linearity, precision, accuracy, specificity, and stability under various stress conditions.[7]

The successful validation of this HPLC method will provide a reliable analytical tool for the quality control of Kimmtrak Injection, ensuring its consistent efficacy and safety for clinical use. This study not only contributes to the scientific literature on Tebentafusp analysis but also underscores the importance of robust analytical methods in maintaining the high standards of pharmaceutical quality assurance.[8]

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II. MATERIALS AND METHOD

API and formulation received from Rainbow Pharma labs Hyderabad. The chemicals used in this study included KH_2PO_4, peroxide, methanol, sodium hydroxide, phosphoric acid, and hydrochloric acid. Drugs: The primary drug analyzed was Tebentafusp (TEBN). Parenterals: The product used was Kimmtrack Injection, which contains 100 mcg/0.5ml of Tebentafusp, manufactured by Immunocore Ltd, UK. Apparatus: The equipment utilized included an HPLC system and a photodiode array detector, both from Waters Alliance.

MOBILE PHASE:

A solution containing a 50% mixture of KH2PO4 buffer and Methanol was exposed to ultrasonic treatment in a water bath for a duration of 10 minutes. The resultant mixture was subsequently filtered using vacuum filtering via a 0.45 micron filter.

DILUENT:

As a diluent, mobile phase blend combination solvents were used.

PREPARATION OF MOBILE PHASE:

Add 1000ml of high-performance liquid chromatography (HPLC) water to a 1000ml beaker. Then, combine it with potassium dihydrogen phosphate (KH2PO4) at a concentration of 1M (136.09gms). Lower the pH to 4.6 by using ortho-phosphoric acid (OPA) to make it more acidic.

Utilize a 500ml solution of KH2PO4 and 500ml of Methanol as the mobile phase. The materials are combined and subjected to sonication for a duration of 20 minutes.[9,10]

PREPARATION OF THE TEBENTAFUSP AND SAMPLE SOLUTION:

PREPARATION OF STANDARD SOLUTION:

Precisely measure and transfer a quantity of 50 milligrams of Tebentafusp into a volumetric flask with a capacity of 100 milliliters. Combine 10 milliliters of Methanol and subject it to sonication for 10 minutes, or alternatively, aggressively shake it for 5 minutes. In the end, dilute the mixture with water.

Transfer the solution stated earlier into a 1ml bottle and then transfer it into a 10ml volumetric flask. Thoroughly blend the solution with water until it reaches the desired volume.

PREPARATION OF SAMPLE SOLUTION:

Accurately pipette 2.5ml of combined injection dosage form containing 100mcg/0.5ml of tebentef in to 10ml volumetric flask and the volume was made up with diluent to give a final concentration of 50mcg/ml.[11]

ASSAYING CONDITIONS FOR LIQUID CHROMATOGRAPHIC DETERMINATION OF TEBN:

STOCK TEBN SOLUTION:

A concentrated solution of TEBN (TEBN – 500 µg /ml) was prepared by dissolving 50 mg of TEBN in 100 ml of the selected solvent.

WORKING TEBN SOLUTIONS:

A functional TEBN solution (TEBN – 50 µg /ml) was created by dissolving 1 ml of the concentrated TEBN solution (TEBN – 500 µg /ml) in 10 ml of the chosen diluent.

LINEARITY TEBN SOLUTIONS:

To achieve linearity, a 0.5 ml portion of a stock TEBN solution (with a concentration of 50 µg/ml) was mixed with 9.5 ml of the chosen diluent to obtain a final concentration of 25 µg/ml. To achieve a linear relationship, a solution with a concentration of 37.5 µg/ml of TEBN was prepared by mixing 0.75 ml of a TEBN stock solution (with a concentration of 50 µg/ml) with 9.25 ml of the necessary diluent. To achieve a solution with a concentration of 50 µg/ml, 1.0 ml of a TEBN solution with the same concentration was mixed with 9.0 ml of the specified diluent.In order to achieve linearity, the procedure involved combining 1.25 ml of a concentrated TEBN solution (with a TEBN concentration of 50 µg/ml) with 8.75 ml of the specified diluent. The outcome was a concluding concentration of 62.5 µg/ml. A solution was

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prepared by mixing 1.50 ml of a stock TEBN solution (TEBN - 50 µg/ml) with 8.50 ml of a specified diluent, resulting in a final concentration of 75 µg/ml.[12]

VALIDATION EVALUATION OF DEVELOPED METHOD:

An evaluation was conducted on the analytical methodology used to measure the amount of TEBN in Kimmtrak Inj. The aim was to ascertain all validation factors in accordance with the standards established by the International Conference on Harmonization.

CONTENT ASSAY OF TEBNIN KIMMTRACKINJECTIONS:

Accurately pipette 2.5ml of combined injection dosage form containing 100mcg/0.5ml of tebentef in to 10ml volumetric flask and the volume was made up with diluent to give a final concentration of 50mcg/ml.

Ten microliters of TEBN Injection solution were injected into a Column Thermo solar C18 with dimensions of 150*4.6 and a particle size of 3.5 micrometers, utilizing pressure. We conducted a comprehensive analysis of the TEBN content in Kimmtrak using the specified parameters in the chromatographic estimation of TEBN.

TEBN STABILITY EVALUATION:

The stability of TEBN was tested by subjecting it to the conditions listed below.

S. No.	Name of the sample	Period of exposure	Physical appearance
1	Mother / control (as such sample)	-	Clear , No change
2	Solution in 2N HCl at 60 °C	Refluxed for 30 min	Clear , No change
3	Solution in 2N NaOH at 60 °C	Refluxed for 30 min	Clear , No change
4	Solution in water at 60 °C	6 hours	Clear , No change
5	Solution in 20 % H_2O_2 at 60 °C	Refluxed for 30 min	Clear , No change
6	Solution under dry heat, 105 °C	6 hours	Clear , No change
7	UV exposed sample	200 Watt hours/m^2 1.2 million lax hrs	Clear , No change

Stability of TEBN in 0.1N HCL:

After subjecting the 100-ml container to 30 minutes of vigorous sound wave treatment, a mixture was prepared by combining 10 ml of concentrated Injection solution (TEBN: 50 µg/ml) with 10 ml of 0.1N hydrochloric acid. After a period of 30 minutes, an adequate amount of a suitable diluent was added to the flask, resulting in a final volume of 100 ml. After administering the suitable treatment, a 10 microliter injection solution was put into the Thermo C18 column. The TEBN Injection solution's composition was analyzed following the guidelines specified in the "TEBN ASSAYING CONDITIONS" section after being treated with 0.1N HCl.[13]

Stability of TEBN in 0.1N NaoH:

The TEBN stock solution, which had a concentration of 50 µg/ml, was dissolved in 10 ml of 0.1N NaOH using ultrasonication for 30 minutes in a 100-ml flask. After a duration of 30 minutes has passed, add 100 ml of an appropriate diluent to a flask in order to reach a total volume of 100 ml. Following appropriate preparation with 0.1N NaOH, a 10-liter quantity of Injection solution was introduced into a Thermo C18 column. Examined the chemical composition of TEBN in a TEBN Injection solution that was subjected to treatment with 0.1N NaOH, in accordance with the guidelines outlined in the "TEBN TESTING CONDITIONS" section.

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Stability of TEBN in 30% peroxide:

As an initial step, we utilized 30 minutes of ultrasonication to aid in the dissolving of the TEBN Injection solution (10ml) in a 30 percent peroxide solution. After a duration of thirty minutes, continuing to decrease the concentration of the material by adding enough solvent to reach a volume of 100 milliliters. An appropriate volume of Injection solution (30% peroxide) was introduced onto the Thermo C18 column (10 mL). An analysis was conducted on a 30 percent peroxide solution that had undergone treatment with peroxide to ascertain the existence of TEBN, in accordance with the guidelines provided in the "TEBN ASSAYING CONDITIONS" section.[14]

Stability of TEBN in sun light:

In order to aid the mixing of the TEBN Injection solution (10ml) with a 30 percent peroxide solution, we utilized 30 minutes of ultrasonication as an initial step. After a duration of thirty minutes, proceed to decrease the concentration of the material by adding an adequate amount of solvent to reach a volume of 100 milliliters. The Thermo C18 column (10 mL) was treated with an appropriate quantity of Injection solution (30% peroxide). An analysis was conducted on a solution comprising 30% peroxide that had undergone peroxide treatment. The analysis aimed to ascertain the existence of TEBN, in accordance with the guidelines provided in the "TEBN ASSAYING CONDITIONS" section.[15]

Stability of TEBN at 105^{o}C:

The TEBN Injection solution, with a concentration of 50 µg/ml, was subjected to direct sunlight for a period of six hours. After the specified time period, the bottle held a total volume of 100 ml.

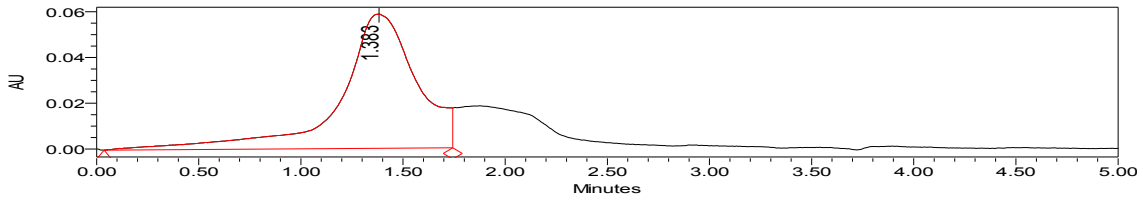
After being exposed to sunlight for six hours, a quantity of ten milliliters of Injection solution was put onto a Thermo C18 column. The TEBN content of an Injection solution treated with a 30% peroxide solution was evaluated using the prescribed conditions outlined in the "TEBN ASSAYING CONDITIONS".[16]

III. RESULTS AND DISCUSSION

DEVELOPMENT AND TRAILS:

Trail:1

Mobile Phase : KH_2PO_4: Methanol (60:40)
Column : inertsil, C18, 250X4.6mm, 3.5µm
Flow Rate : 0.8 ml/Min
Column Temperature : 25˚C
Sample Temperature : 25˚C
Volume : 10µl
Run time : 10min
Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		1.383	1529673	100.00	58688	BV			96

CONCLUSION Peak shape is not good, Merged Peak was observed; USP plate count was found to be very less;. Due to all these reasons we'll perform another trial.

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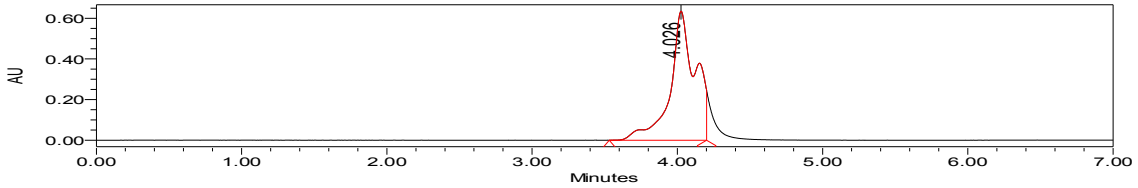
Trail-2

Mobile Phase : KH_2PO_4: Methanol (60:40)
Column : X-BRIDGE,C18, 250X4.6mm, 3.5µm
Flow Rate : 0.8 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA

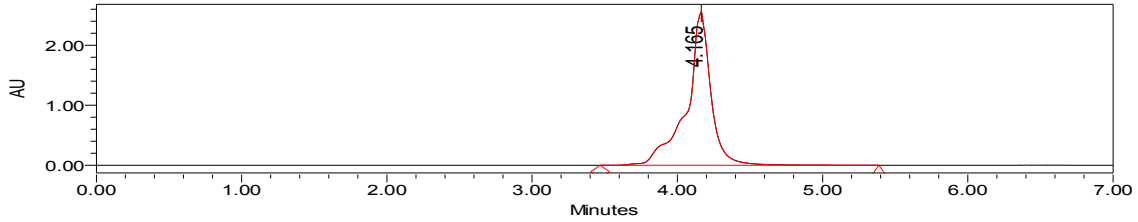


	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		4.026	7600570	100.00	634902	BV		2.1	4854

CONCLUSION: Peak shape is not goo,Merged peak observed,Tailing is not with in the limit; Due to all these reasons we'll perform another trial.

Trail-3

Mobile Phase : KH_2PO_4: Methanol (70:30)
Column : X-BRIDGE,C18, 250X4.6mm, 3.5µm
Flow Rate : 1 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		4.165	29695011	100.00	2552700	VB		0.78	6079

CONCLUSION: Peak shape is not good; Due to all these reasons we'll perform another trial.

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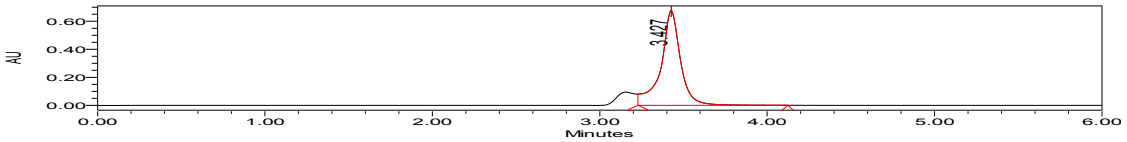
Trail-4

Mobile Phase : KH_2PO_4: Methanol (70:30)
Column : PHENOMENICS,C18, 250X4.6mm, 3.5µm
Flow Rate : 1ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA

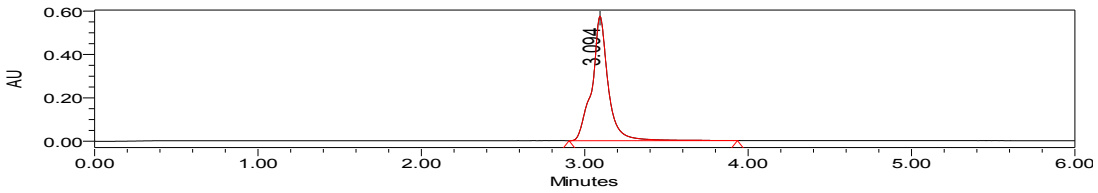


	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.427	5513696	100.00	675578	VB		1.2	5613

CONCLUSION: Peak shape is not good ;Due to all these reasons we'll perform another trial.

Trail-5

Mobile Phase : KH_2PO_4: Methanol (50:50)
Column : THERMOSTAT,C18, 250X4.6mm, 3.5µm
Flow Rate : 1 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.094	3976751	100.00	573347	BB		1.06	6712

CONCLUSION: PEAK SHAPE IS NOT GOOD; Due to all these reasons we'll perform another trial.

Trail-6

Mobile Phase : KH_2PO_4: Methanol (50:50)
Column : THERMOSTAT,C18, 250X4.6mm, 3.5µm
Flow Rate : 1 ml/Min
Column Temperature : 25°C

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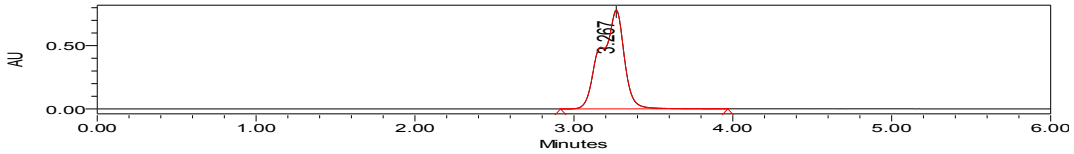
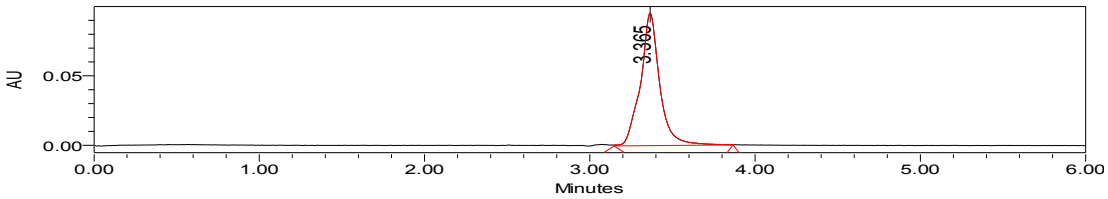
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.267	8053207	100.00	780261	BB		0.83	482

COCNCLUSION: Peak shape is not good ; Due to all these reasons we'll perform another trial.

Trail-7
Mobile Phase : KH_2PO_4: Methanol (60:40)
Column : THERMOSTAT,C18, 250X4.6mm, 3.5µm
Flow Rate : 1 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		3.365	771624	100.00	95234	VB		1.07	4892

CONCLUSION: PEAK SHAPE IS NOT GOOD; Due to all these reasons we'll perform another trial

Trail-8
Mobile Phase : KH_2PO_4: Methanol (50:50)
Column : THERMO,C18, 150X4.6mm, 3.5µm
Flow Rate : 1 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA

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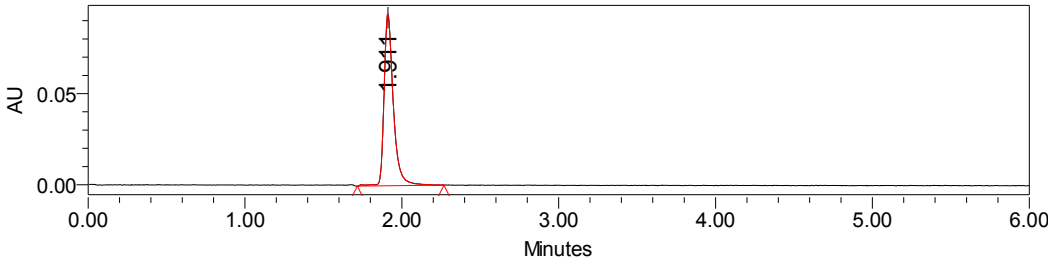
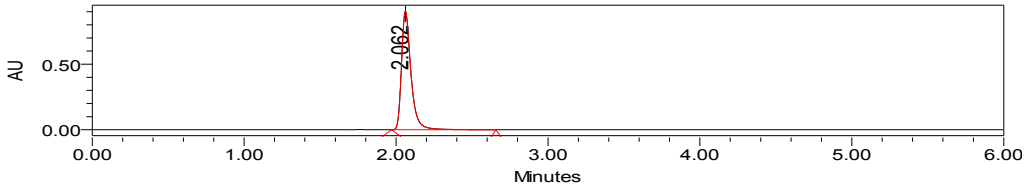


	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		1.911	388715	100.00	94280	BB		1.46	5615

CONCLUSION: Peak shape is not good we can do another trail.

Trail-9
Mobile Phase : KH_2PO_4: Methanol (60:40)
Column : THERMO,C18, 150X4.6mm, 3.5µm
Flow Rate : 0.8 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA



	Name	Retention Time	Area	% Area	Height	Int Type	USP Resolution	USP Tailing	USP Plate Count
1		2.062	3909352	100.00	904430	VB		1.44	5790

CONCLUSION: peak shape is good .

Trail-10
Mobile Phase : KH_2PO_4: Methanol (60:40)
Column : THERMO,C18, 150X4.6mm, 3.5µm
Flow Rate : 1 ml/Min
Column Temperature : 25°C
Sample Temperature : 25°C
Volume : 10µl
Run time : 10min
Detector : PDA

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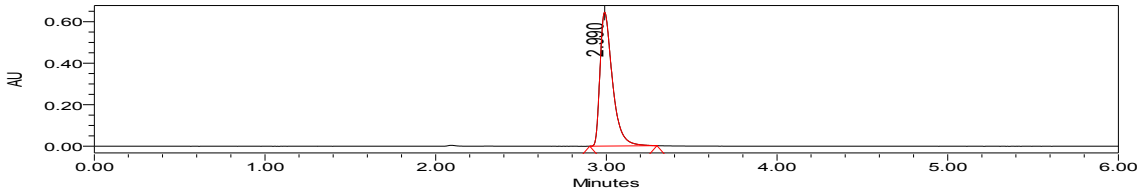

	Name	Retention Time	Area	% Area	Int Type	USP Resolution	s/n	USP Tailing	USP Plate Count
1		2.990	3260037	100.00	BB		4147	1.67	8568

CONCLUSION:THE SHAPE IS GOOD AND ALL SYSTEM SUITABILITY PARAMETERS ARE PASSED.

V. VALIDATION OF DEVELOPED METHOD BY UTILISING ICH

LINEARITY:

To achieve linearity, a solution of TEBN was prepared by combining 0.5 ml of a TEBN solution with a concentration of 50 µg/ml (from the stock) with 9.5 ml of the needed diluent. The resulting solution had a concentration of 25 µg/ml.

The solution was prepared by mixing 0.75 ml of a stock TEBN solution, with a concentration of 50 µg/ml, with 9.25 ml of the necessary diluent.

To achieve a solution with a concentration of 50 µg/ml, 1.0 ml of a TEBN solution with the same concentration was mixed with 9.0 ml of the necessary diluent.

In order to achieve linearity, the method consisted of melting 1.25 ml of a stock TEBN solution (with a concentration of 50 µg/ml) by combining it with 8.75 ml of the specified diluent. The outcome was a concluding concentration of 62.5 µg/ml.

To achieve a linear solution, 1.50 ml of the stock TEBN solution (TEBN - 50 µg/ml) was mixed with 8.50 ml of the necessary diluent, resulting in a concentration of 75 µg/ml.

The collected results demonstrated a significant level of linearity while analyzing TEBN.

Table 1:TEBNENTAFresponse peak area and concentration

TEBNENTAF	
Response peak area	**µg/ml amount**
1562606	25
2360967	37.50
3158263	50.00
3940901	62.5
4747366	75

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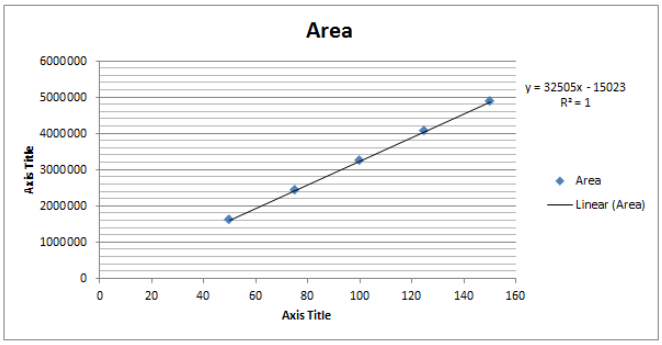
Figure 18:TEBNENTAF calibration curves



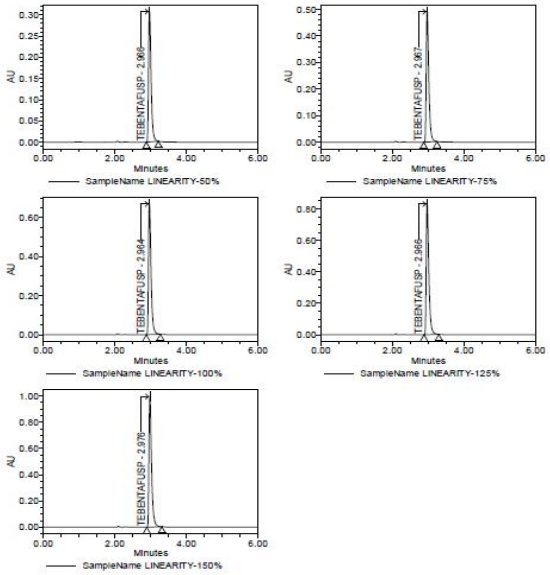


Fig 19:TEBNENTAFresponse peak area and concentration

LIMIT OF DETECTION:

Determined using technique of standard deviation with formula:

Limit of detection = 3.3 × deviation of drug response peak area/slope of drug calibration curve

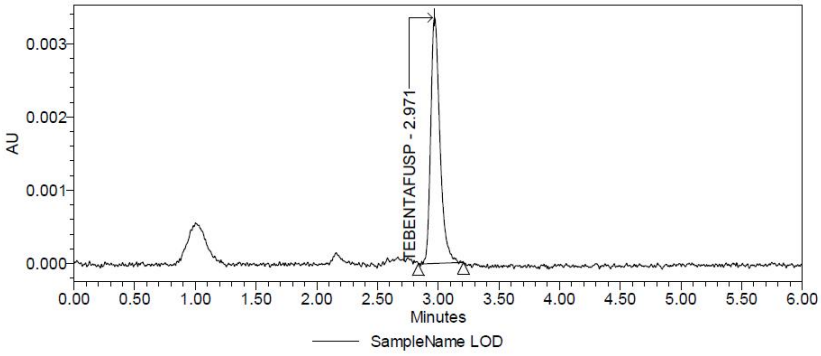


Figure 20: Reading TEBNchromatogram – sensitivity – limit of detection

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Limit of detection for **TEBN**: 0.076 µg/ml
signal / noise ratio for **TEBN** at µg/ml is 1963.3
The data gathered proved method have enough sensitivity for TEBN analysis.

LIMIT OF QUANTITATION:
Determined using technique of standard deviation with formula:
Limit of quantitation = 10 × deviation of drug response peak area/slope of drug calibration curve

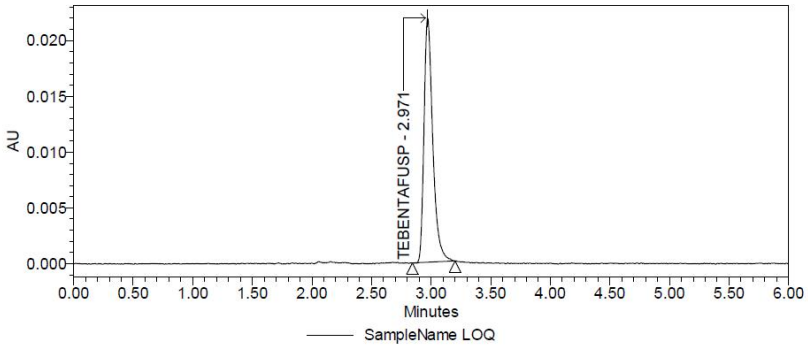

Figure 21: Reading TEBNchromatogram – sensitivity – limit of quantitation

Limit of quantitation for **TEBN**: 0.255 µg/ml
Ratio of signal to noise for **TEBN** at µg/ml is 1963.3
The data gathered proved method have enough sensitivity for TEBN analysis.

PRECISION:
10 microliters of TEBN solution, which had a concentration of 50 micrograms per milliliter, were injected into a C18 column of 150 x 4.6 mm and with a particle size of 3.5 micrometers. The injection was administered on six occasions. The data was examined utilizing the criteria specified in the "TEBN ANALYSIS CONDITIONS" section. The peak values of the TEBN responses were determined. The standard deviation and relative percent standard deviation were computed for the peak areas of six TEBN responses. The gathered data confirmed the accuracy of the procedure in TEBN analysis.

Table 7: Precision investigation readings for TEBNENTAF

Response peak area	
TEBNENTAF	
3232609	**Mean response peak area**
3237413	3234811
3249176	**Standard response Deviation**
3225747	8719.7
3226313	**RSD**
3237607	**0.3**

ACCURACY:
Six injections of a TEBN solution, each containing 10 microliters and having a concentration of 50 micrograms per milliliter, were made into a C18 column measuring 150 x 4.6 mm and containing particles with a size of 3.5 micrometers. The data was examined based on the criteria specified in the "TEBN COMBINATION ANALYSIS CONDITIONS" section. The specified assay percentages for TEBN were given. The statistical parameters of mean, standard deviation, and relative percent standard deviation were computed for six TEBN injections. The results validated the precision of the current TEBN analysis methodology.

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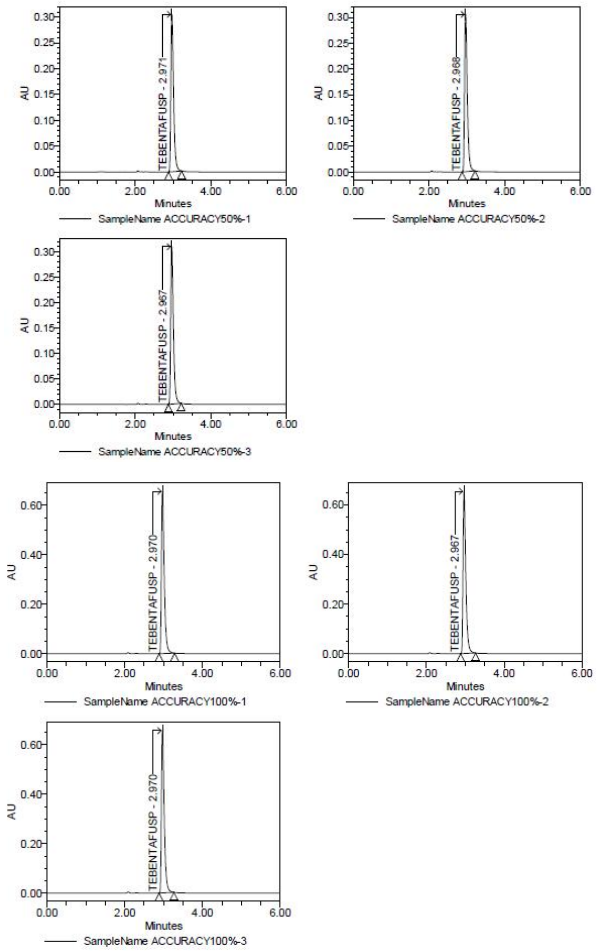
Table 8: Accuracy investigation readings for TEBNENTAF

µg/ml amount considered	µg/ml amount quantified	Assay percent	
TEBNENTAF			
24.750	24.51	99	
24.750	24.73	100	**Mean percent assay**
24.750	24.60	99	100
49.500	49.45	100	**Standard assay Deviation**
49.500	49.57	100	0.6
49.500	49.68	100	**RSD**
74.250	74.51	100	0.6
74.250	74.65	101	
74.250	74.45	100	



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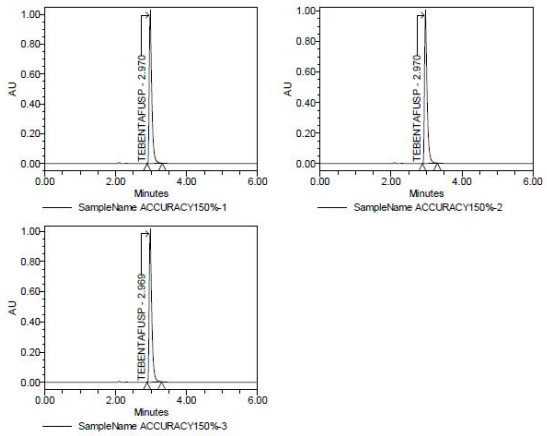

SELECTIVITY:

The selectivity was assessed using the conventional addition method to get the percentage of TEBN recovered. The TEBN stock solution was enhanced with TEBN concentrations of 50%, 100%, and 150% at concentrations of 25 µg/ml, 50 µg/ml, and 75 µg/ml, respectively. The collected samples underwent analyzed on three distinct occasions, adhering to the parameters specified in the paper titled "TEBN ASSAYING CONDITIONS".

µg/ml amount considered	µg/ml amount quantified	Recovered percent	
50% additional			
24.750	24.51	99	**Mean percent recovered** 99
24.750	24.73	100	
24.750	24.60	99	
100% additional			
49.500	49.45	100	**Mean percent recovered** 100
49.500	49.57	100	
49.500	49.68	100	
150% additional			
74.250	74.51	100	**Mean percent recovered** 100
74.250	74.65	101	
74.250	74.45	100	

ROBUSTNESS:

The "TEBN ASSAYING CONDITIONS" section underwent slight modifications to the parameters used to assess the TEBN solution, mostly involving the adjustment of the TEBN concentration to 50 µg/ml. The device values were considered appropriate for TEBN. The effectiveness of TEBN analytical approaches was demonstrated using the acquired data.

Modification one:

Methanol ratio optimized was 50% volume

Modified values were 60% (comp 1) and 40% (comp 2)

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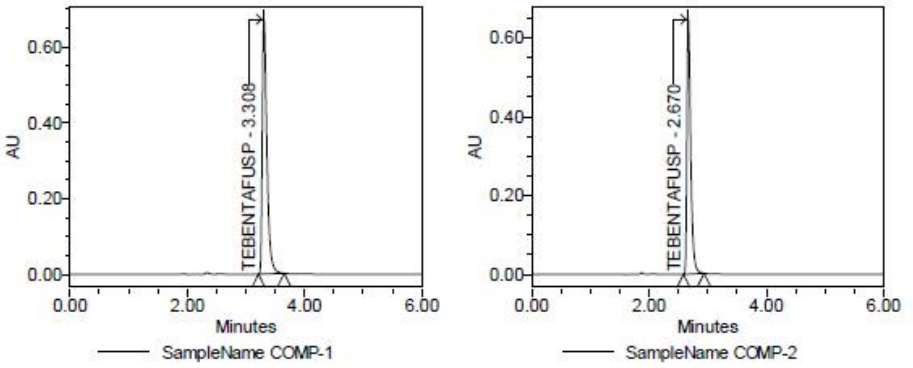

Modification two:

Flow optimized : 1.0 ml per min

Modified values were 0.9 (flow 1) and 1.1 (flow 2)

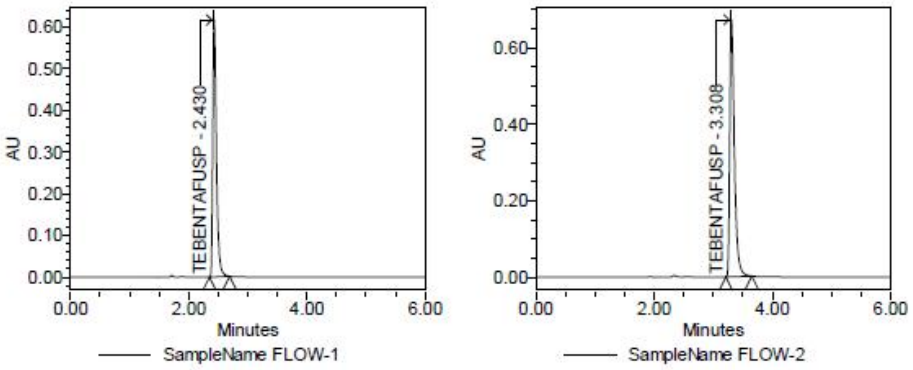

Figure 25: Readings of suitability testing and chromatograms of robustness (flow modification)

Modification three:

nm optimized was 330

Modified values were 325(nm 1) and 335 (nm 2)

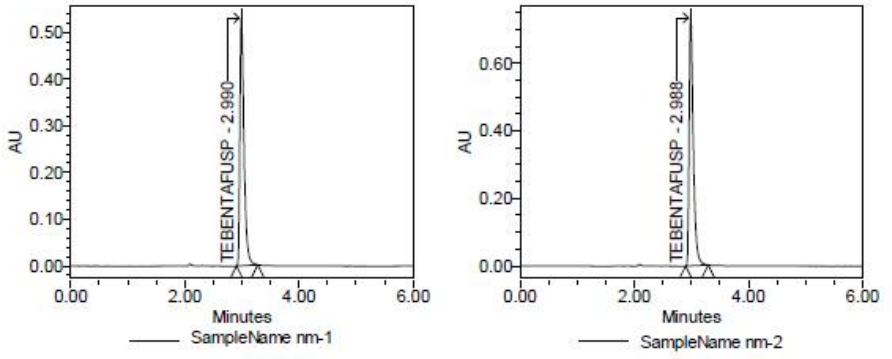

Figure 26: Readings of suitability testing and chromatograms of robustness (nm modification)

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Modification four:
pH optimized was 4.6
Modified values were 4.4 (pH 1) and 4.8 (pH 2)

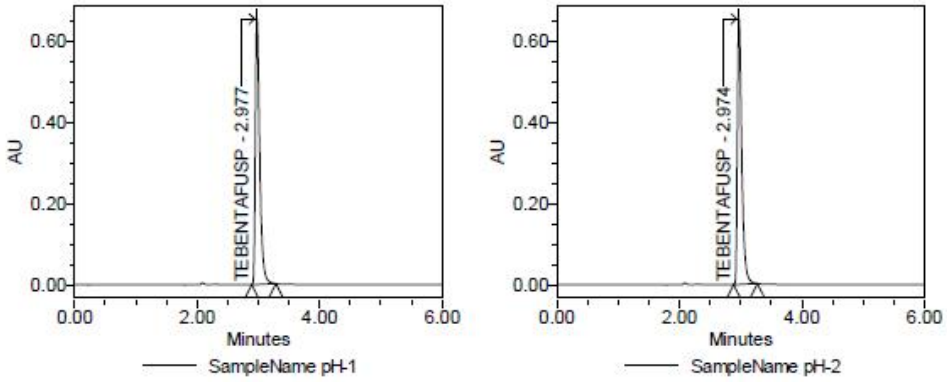

Figure 27: Readings of suitability testing and chromatograms of robustness (pH modification)

STABILITY OF TEBN:
The stability of TEBN was evaluated by submitting it to different conditions, such as exposure to 0.1 N HCl, 0.1 N NaOH, peroxide, sunshine, and a temperature of 105°C. The findings of these inquiries are displayed in the table provided below.

	TEBN	
Drug exposed	**Response Area**	**% Assay**
Acid	2885636	88.30
Base	3011662	92.16
Peroxide	3095557	94.73
Heat	2969651	90.87
Sunlight	3105479	95.03
Untreated	3208982	98.20

TEBN stability direction:
Acid>Heat >Base >Peroxide >Sunlight

STABILITY INDICATING NATURE AND SPECIFICITY:
The chromatograms of TEBN stability reports are shown after being exposed to 0.1 N HCl, 0.1 N NaOH, peroxide, sunshine, and a temperature of 105 degrees Celsius. The resolution between TEBN and ETC, as evidenced by the disparity in RT values, is outstanding and satisfactory in deteriorated compounds. The findings indicated that the approach is dependable and precise for TEBN investigations.

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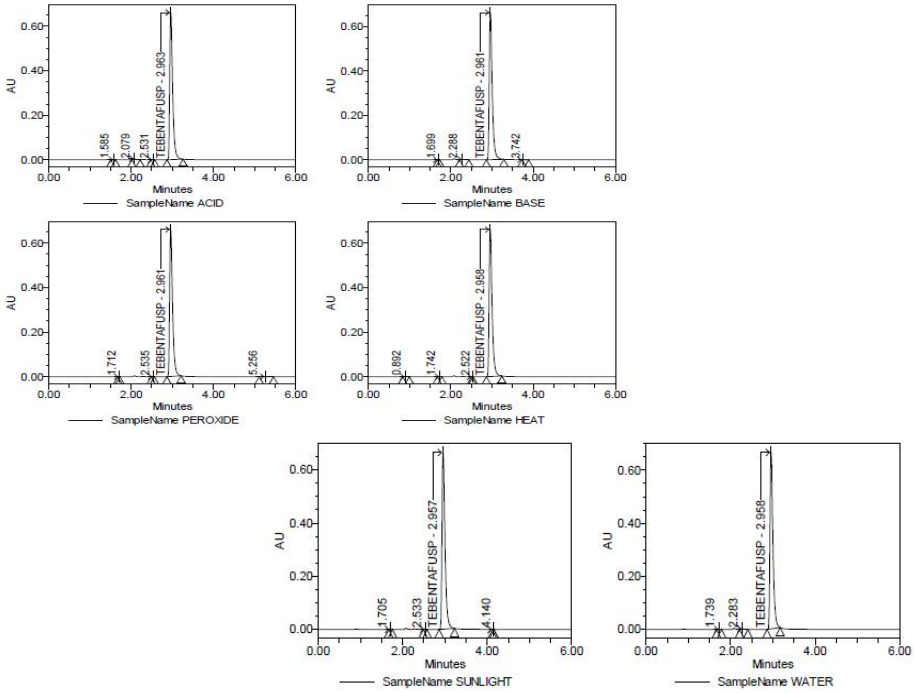

Figure 28: Readings of chromatograms of stability nature and specificity

VI. CONCLUSION

In this study, we successfully developed and validated a robust high-performance liquid chromatography (HPLC) method for the quantitative analysis of Tebentafusp in Kimmtrak Injection. Utilizing a photodiode array detector (PDA), the optimized chromatographic conditions ensured precise and accurate quantification of the active pharmaceutical ingredient (API) while maintaining excellent resolution and reproducibility. Stability tests confirmed the method's reliability under various stress conditions, demonstrating its applicability for routine quality control and stability assessment. The method validation parameters, including linearity, precision, accuracy, specificity, and sensitivity, met the stringent criteria set by regulatory guidelines, confirming the method's suitability for Tebentafusp analysis in pharmaceutical formulations. This validated HPLC method provides a valuable tool for ensuring the quality and efficacy of Kimmtrak Injection, contributing to the overall safety and therapeutic effectiveness of the treatment.

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