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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Larotrectinib in its Formulations

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ABSTRACT

A stability indicating HPLC method for the quantification of Larotrectinib in capsule form was developed and validated as per the ICH guidelines. Separation and quantification of Larotrectinib was carried out on column Sunsil C_{18} using mobile phase as KH_2PO_4 and methanol in 1:1 ratio. Larotrectinib was eluted at 3.432 minutes. Linearity was observed in between 50-150 µg/ml. LOD and LOQ were found to be 0.065 µg/ml and 0.217 µg/ml respectively. % RSD for the precision of the method was found to be 0.115. Accuracy was well within the regulated limit that is 100.13% and the recovery was found to be to 100.47%. Forced degradation analysis was carried out on Larotrectinib which established stability indicating power of the developed method.

Keywords: HPLC, Larotrectinib, Method development, ICH Guidelines.

INTRODUCTION

A medication, Vitrakvi (Larotrectinib), IUPAC term (3S)-N-{5-[(2R)-2-(2,5-difluorophenyl)-1-pyrrolidinyl]pyrazolo[1,5-a]pyrimidin-3-yl}-3-hydroxy-1-pyrrolidinecarboxamidesulphate was authorized by US Food & Drug Administration (FDA) to manage tumors with particular genetic modification regardless of cancer type. Vitrakvi (Larotrectinib) is authorized for managing adults and children having solid tumors which give positive test for NTK genes[1]. Tumors with that kind of genetic modification are not prevalent but it can be seen in salivary gland cancer, pulmonary cancer and sarcoma in tissue. Tumors which have distributed or not surgically removed and have grew up during earlier medicines must be treated with Larotrectinib. Present work is aimed to develop a new, efficient and reproducible HPLC method for

the analysis of Larotrectinib. The developed method is validated according to ICH guidelines for various parameters specified in guidelines[2,3,4]. Separation and quantification of Larotrectinib was carried on column Sunsil C_{18} using mobile phase as KH_2PO_4 and methanol in 1:1 ratio. Larotrectinib was eluted at 3.432 minutes. The method was validated for parameters such as specificity, linearity, precision, accuracy, system suitability, limit of detection , limit of quantification and robustness.

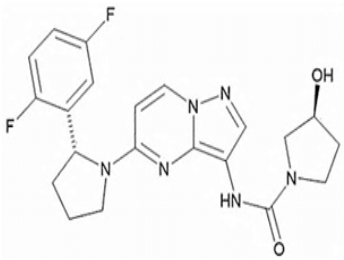

Fig. 1. Larotrectinib structure

MATERIAL AND METHODS

Table 1: Instruments used

Instrument	Model	Description
HPLC system	2695 model	Water alliance
Column	Sunsil C_{18}	250 mm×4.6 mm, 5µm
Software	Empower	Water alliance
Photodiode array	2998 Model	Water alliance

Table 2: Drug, chemicals and solvents used

Material	Source
Larotrectinib	Octapharma pvt.ltd, India
Dipotassium hydrogen phosphate	Sd Fine-Chem Ltd, India
Hydrochloric acid	Sd Fine-Chem Ltd, India
Sodium hydroxide	Sd Fine Chem Ltd, India
Hydrogen peroxide	Sd Fine Chem Ltd, India
Phosphoric acid	Sd Fine Chem Ltd, India
Methanol	Merck specialities Ltd, India

Table 3: HPLC method conditions

Column with temperature	Ambient
pH units	4.3 units
Injection vol sample	10 µl
Column rate of flow	1ml/min
Run time	5 minutes
wave length of detection	228 nm

Preparation of mobile phase

KH_2PO_4 with strength 0.1 M and methanol mixed in 50:50 v/v ratios and the pH was fixed to 4.3 with the aid of phosphoric acid. As both mobile phase and diluent, this solvent mix was used.

Preparation of stock solution

100 mg of standard Larotrectinib was dissolved in 100 mL volume of mobile phase. Stock Larotrectinib solution-1000 µg/ml concentration.

Assay methodology

Larotrectinib capsules (label claim – 100 mg/capsule) were emptied. Capsule powder weight equivalent to 100 mg Larotrectinib was taken to standard flask (100 mL). 25 mL mobile phase was added and dissolved and make up the volume to 100 mL. Concentration of Larotrectinib in solution (stock capsule solution) was 1000 µg/ml. 1 mL stock capsule solution is mixed with 9 mL of diluent. Then concentration of Larotrectinib in this capsule solution was 100 µg/mL. This capsule solution was analyzed employing proposed HPLC conditions. The Larotrectinib amount in capsule was calculated with acquired peak areas.

ASSAY%:

$$\frac{\text{Area/Abs of sample}}{\text{Area/Abs of standard}} \times \frac{\text{Weight of Standard}}{\text{Dilution of Standard}} \times \frac{\text{Dilution of Sample}}{\text{Weight of Sample}} \times \frac{\text{Average Weight}}{\text{L.C}} \times \text{Purity of STD}$$

Table 4: Optimized chromatographic conditions

Mobile Phase	Na_2HPO_4 (50 mL and methanol (50 mL)
pH of mobile phase	4.3
Chromatographic column	Phenomenex, C_{18}, length – 250 mm, Identification -4.6 mm, particle -5 µm
Flow Rate	1.0 ml/min
Injection Volume	10 µl
Temperature of column	25°C
Detection wavelength	228 nm
Time of run	5 minutes

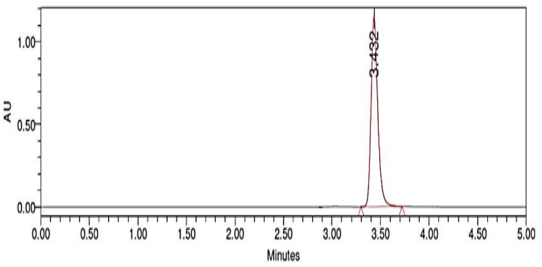

Fig. 2. Chromatogram with optimized conditions

Assay of formulation

Standard and sample solutions were injected separately into the system and chromatograms were recorded. The drug present in sample was calculated using mentioned formula.

Table 5: Assay of formulation

S.No	% Assay
1	99
2	99
3	99
4	99
5	99
6	100
Average assay:	99
Standard deviation	0.11
%RSD	0.12

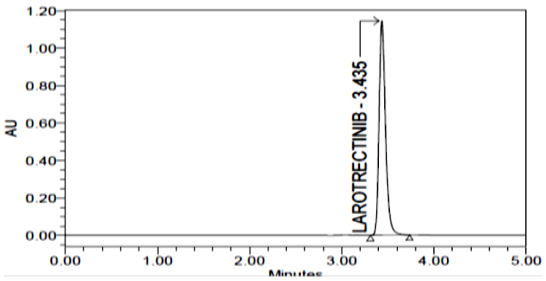

Fig. 3. Sample chromatogram

Method validation[5,6,7]

Selectivity

Interference of blank diluent, placebo and excipient in capsule solution was assessed. Analysis was done on blank diluent, placebo and excipient in capsule solution and compared with Larotrectinib standard (100 µg/mL). Interference peaks were not noticed at the retention time of Larotrectinib in chromatograms of blank diluent, placebo and capsule solution. This clearly showed ability of method to selectively analyze Larotrectinib.

System suitability

To test system effectiveness 10 µl of Larotrectinib standard (100 µg/mL) injected five times. Result of system suitability (Plate count, RSD of peak area, retention time and tailing factor) were computed. The results were well within the limits of ICH prescribed.

Prescribed limits

- More than 2000-Plate count
- Less than or equal to 2%- Peak area RSD
- Retention time- reliably less
- Less than or equal to 2% - Tailing factor

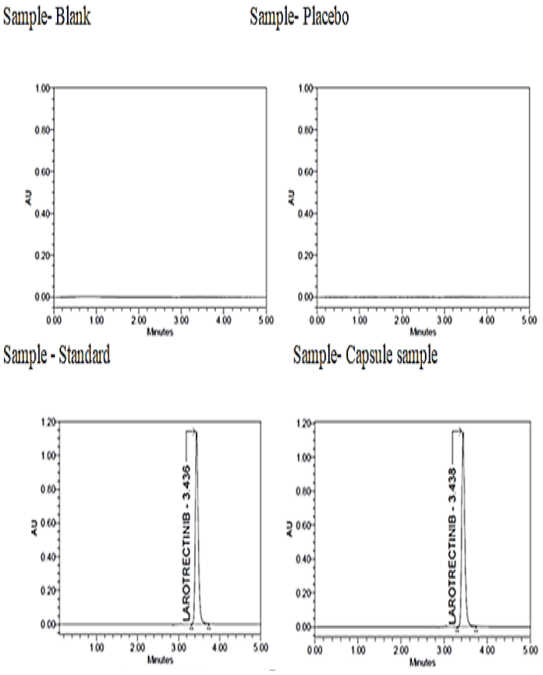

Fig.4. Selectivity evaluation chromatograms

Table 6: Results for system suitability

Peak Name: Larotrectinib					
S.No	Sample Name	Rt	Area	USP Plate Count	USP Tailing
1	Sample 1	3.436	5547055	12095	1.29
2	Sample 2	3.425	5549323	12105	1.29
3	Sample 3	3.434	5538730	12289	1.29
4	Sample 4	3.436	5546059	12179	1.29
5	Sample 5	3.435	5536725	12211	1.29
Mean			5543578.2		
%RSD			0.1		

Linearity

Five calibration samples of Larotrectinib were made (50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL and 150 µg/mL) and injected into chromatographic system. Plot the graph of measured Peak area Vs. concentration and calculated the regression coefficient. Good linear relationship is observed with correlation coefficient of 0.9998.

Table 7: Results of linearity

%Concentration with respect to target conc.	Larotrectinib area	Larotrectinib conc.(µg/ml)
50	2767034	50
75	4153769	75
100	5539444	100
125	6921686	125
150	8318378	150

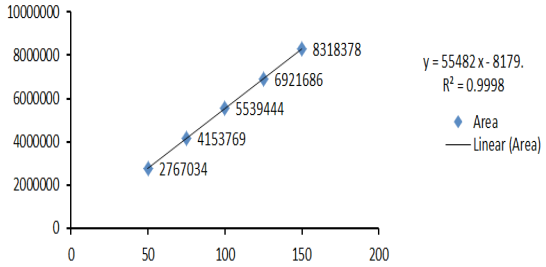

Fig. 5. Larotrectinib linearity graph

LOD and LOQ

The concentration of Larotrectinib with signal to noise ratio 3:1 is taken as LOD and 10:1 as LOQ.

Table 8: Signal to noise details in LOD and LOQ

S.No	Sample name	Rt	Area	S/N ratio
1	LOD	3.531	31853	3.96
2	LOQ	3.537	29391	10.28

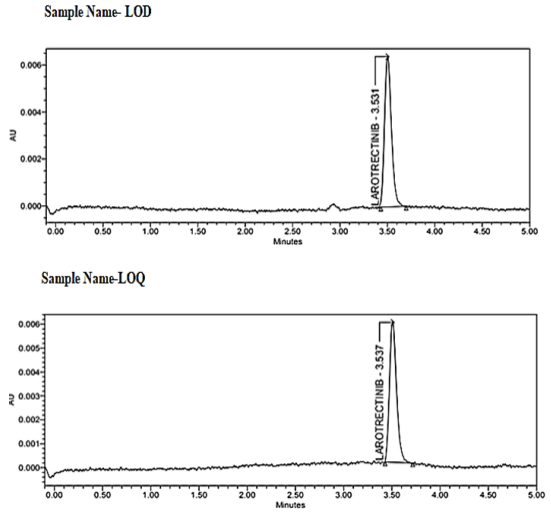

Fig. 6. Sensitivity evaluation chromatograms

Precision

Standard Larotrectinib solution is injected (n= 6 times) in the system. Measured mean area and RSD for 6 injections. The RSD for area of 6 injections is lower than 2%, which shows good precision.

Table 9: Results of precision

Larotrectinib-100 mg	
S.No	Area
1	5529736
2	5525264
3	5523125
4	5523255
5	5530329
6	5539924
Average area	5528803
STD	0.114
%RSD	0.115

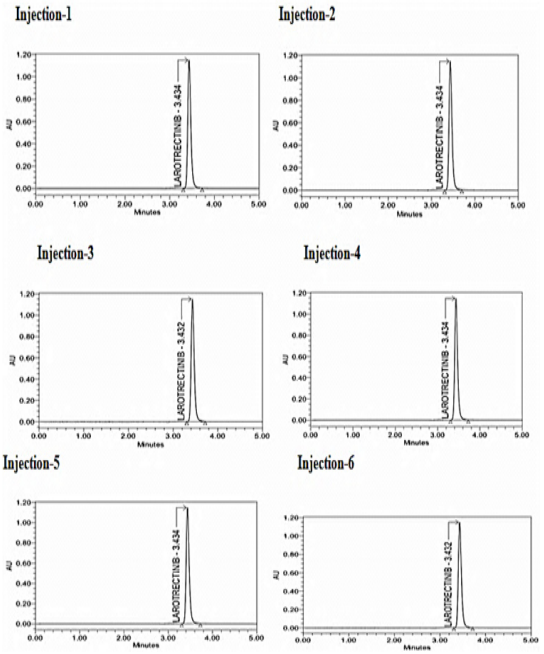

Fig. 7.Precision evaluation chromatograms

Accuracy

Accuracy was determined through analysis (n = 3) for different three concentrations (49.5 µg/mL - 50% level; 99 µg/mL - 100% level; 148.5 µg/mL-150% level) of Larotrectinib spiked to already analyzed capsule solution. Mean recovery at different three concentrations were computed. The values are nearby 100%, which shows good accuracy.

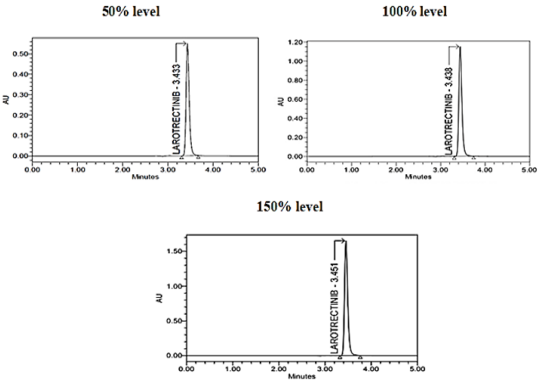

Fig. 8. Accuracy evaluation chromatograms

Robustness

Robustness was checked by determining parameters for system suitability by making small but deliberate variations in assay conditions as given.

- Flow 1: 0.9 mL/min

- Flow 2: 1.1 ml/min
- Temperature 1: 23°C
- Temperature 2: 27°C
- pH 1: 4.1 unit
- pH 2: 4.5 unit
- Methanol composition at 45% and 55%

Table 10: Results of accuracy evaluation

Level added	Larotrectinib area	µg/ml Larotrectinib added	µg/ml Larotrectinib found	%Larotrectinib recover	Mean
50%	2755478	49.500	49.56	100.11	100.13
50%	2756510	49.500	49.58	100.15	
50%	2755948	49.500	49.57	100.13	
100%	5525880	99.000	99.38	100.39	100.36
100%	5520131	99.000	99.28	100.28	
100%	5527423	99.000	99.41	100.41	
150%	8295365	148.500	149.19	100.46	100.47
150%	8292770	148.500	149.14	100.43	
150%	8298628	148.500	149.25	100.50	

Table 11: Results of robustness evaluation

	Peak name : Larotrectinib			
Sample name	Rt	Area	USP Tailing	USP plate count
Flow-1	2.937	4687232	1.26	11621
Flow-2	3.235	5215096	1.27	11925
Temp-1	4.026	6541959	1.29	12290
Temp-2	4.618	7286964	1.28	13298
Comp-1	2.937	4687232	1.26	11621
Comp-2	4.026	6541959	1.29	12290
pH-1	3.433	5539736	1.28	12220
pH-2	3.435	5545264	1.28	12237

There were no substantial changes to the values. This proves the robustness of the method

Degradation/Stability test for Larotrectinib

Stability check/degradation study of Larotrectinib was carried out using ICH criterion with capsule solution of 1000 µg/ml concentration.

Acid degradation[8-12]

1 mL of stock Larotrectinib solution is mixed with 1 mL 0.1 N HCl followed by sonication for nearly 30 min at 25±2°C temperature. The mixture was made to 10 mL volume by diluent (100 µg/mL). This degraded capsule solution was analyzed employing proposed HPLC conditions. The Larotrectinib amount degraded and remained in capsule was calculated with acquired peak areas.

Ikali degradation[13,14]

1 mL of stock Larotrectinib solution is mixed with 1 mL 0.1 N NaOH followed by sonication for nearly 30 min at 25±2°C temperature. The mixture was made to 10 mL volume by diluent (theoretical Larotrectinib concentration - 100µg/mL). This degraded capsule solution was analyzed employing proposed HPLC conditions. The Larotrectinib amount degraded and remained in capsule was calculated with acquired peak areas.

Peroxide degradation[15,16]

1 mL of stock Larotrectinib solution is mixed with 1 mL of 30% hydrogen peroxide followed by sonication for nearly 30 min at 25±2°C temperature. The mixture was made to 10 mL volume by diluent (theoretical Larotrectinib concentration - 100 µg/mL).

This degraded capsule solution was analyzed employing proposed HPLC conditions. The Larotrectinib amount degraded and remained in capsule was calculated with acquired peak areas.

Thermal degradation[17-19]

Capsule powder weight similar to 100 mg Larotrectinib was placed in petri plate and exposed to 100°C for nearly 6 hours. Cool the sample to 25±2°C temperature and transfer to standard flask (100 mL).To which 25 mL of mobile phase added and dissolved and make up the volume to 100 mL.

1 mL prepared solution is mixed with 9 mL diluent (theoretical Larotrectinib concentration - 100 µg/mL). This capsule solution was analyzed employing proposed HPLC conditions. The Larotrectinib amount in capsule was calculated with acquired peak areas.

Photo degradation[20-23]

Capsule powder weight equivalent to 100 mg Larotrectinib was placed in petri plate and exposed to sunlight for nearly 6 hours. Cool the sample to 25±2°C temperature and transfer to standard flask (100 mL). 25 mL mobile phase added and dissolve the drug through sonication. Mobile phase volume of 75 mL is added and properly mixed. 1 mL of prepared solution is mixed with 9 mL of diluent (theoretical Larotrectinib concentration - 100 µg/mL). This capsule solution was analyzed employing proposed HPLC conditions. The Larotrectinib amount in capsule was calculated with acquired peak areas.

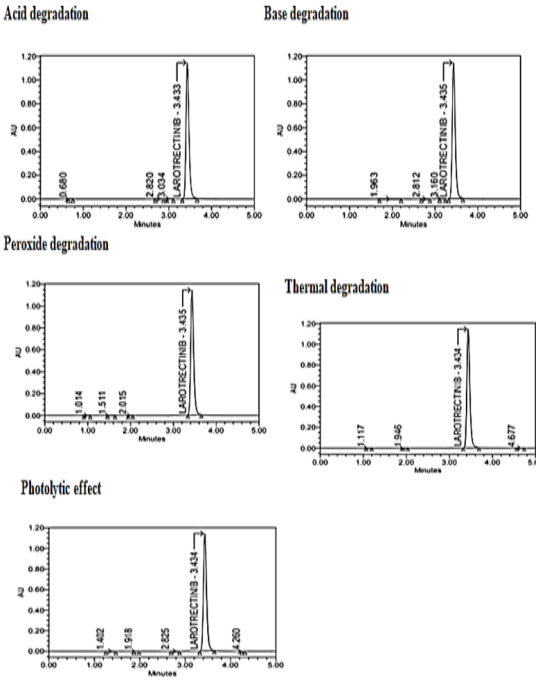

Fig. 9. Specificity/stability indicating evaluation chromatograms

Table 12: Results of Larotrectinib stability evaluation

Condition	Larotrectinib area after degradation	% remained after degradation	% remained after stress applied
Acid	4902388	88.17	11.83
Alkali	5211566	93.73	6.27
Peroxide	5388047	96.9	3.1
Thermal	5020477	90.29	9.71
Photolytic	5296807	95.26	4.74

The generation of separate peaks with distinct retention times with the peak of Larotrectinib showed its degradation. The retention time of additional peaks was completely different from retention time of Larotrectinib which proved specificity and stability indicating power.

DISCUSSION

The method development and validation of Larotrectinib was performed and the results were within the guidelines as mentioned in the standards i.e. the relative standard deviation was found to be not more than 2%, method precision was found to be not more than 2.0%, accuracy was found to be between 98% - 102%, robustness was found to be within the regulated limits.

CONCLUSION

In the present investigation a simple, sensitive and accurate RP-HPLC procedure was developed for evaluation of Larotrectinib in capsule dosage form. Degradation analysis was done and concluded that Larotrectinib is more stable in peroxide and less stable in acid form. From the above studies it was concluded that the proposed RP-HPLC method can be successfully used for the estimation of Larotrectinib in capsule form. This method can be used for the routine analysis in research institutions, QC departments of the pharmaceutical industries.

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Conflicts of Interests

The authors declare that they have no conflict of interest.

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