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RESEARCH ARTICLE

Analytical Method Development and Validation of Selpercatinib in Pure and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

A robust and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of Selpercatinib in pure and pharmaceutical formulations. The method uses a C18 column with a mobile phase of 0.2% trifluoroacetic acid (TFA) and acetonitrile (50:50 v/v) at pH 6.5, detected at 240 nm. Method validation as per ICH Q2 (R1) guidelines demonstrated specificity, linearity (2–10 µg/ml, r^2=0.9995), accuracy (mean recovery 100.61%), precision (%RSD <2%), robustness, and system suitability. The assay of marketed formulations showed 99.68% content, confirming the method's applicability for routine quality control.

Keywords: Selpercatinib, RP-HPLC, Method Development, Validation, ICH Guidelines, Pharmaceutical Analysis.

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INTRODUCTION

Selpercatinib is a selective RET kinase inhibitor used in treatment of RET fusion-positive cancers. Lung cancer is among the most prevalent malignant tumors globally, representing a significant threat to human health and presenting a major challenge to public health. [3], [6], Among the various subtypes of lung cancer, non-small cell lung cancer (NSCLC) is the most prevalent, comprising approximately 85%–90% of all lung cancer cases ,[1,2] Surgical treatment is generally applicable to patients diagnosed at an early stage, while radiotherapy is primarily used to control the growth of localized tumors. Although chemotherapy is widely utilized in advanced cases, its detrimental effects on normal cells can lead to severe side effects, significantly reducing the patient's quality of life. [4, 5] Common targeted drugs currently include EGFR inhibitors, ALK inhibitors, KRAS inhibitors, and RET inhibitors. [7,8,9,10] RET is a transmembrane glycoprotein receptor tyrosine kinase encoded by the RET proto-oncogene, which is located on chromosome 10. [11]

Currently, HPLC technology remains a commonly used method for detecting related substances in active pharmaceutical ingredients (API). [12, 13] Furthermore, researchers, focused on studying the impurities produced during forced degradation of selpercatinib and identifying their structures using liquid chromatography-mass spectrometry. The high-performance liquid chromatography method they established is only used for detecting degradation impurities with higher contents, while neglecting the separation of degradation products with unknown structures and process impurities.[14, 15] Accurate quantification of Selpercatinib in bulk and dosage forms is essential for quality control and therapeutic monitoring. RP-HPLC is a preferred analytical technique due to its sensitivity, specificity, and reproducibility. This study develops a validated, economical RP-HPLC method for Selpercatinib quantification in pure drugs and commercial formulations.

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MATERIALS AND METHODS

Chromatographic Conditions

- Column: C18 (250 × 4.6 mm, 5 µm)
- Mobile Phase: 0.2% TFA and acetonitrile in 50:50 v/v, TFA adjusted to pH 6.5

- Flow Rate: 1.0 mL/min
- Detection Wavelength: 240 nm
- Injection Volume: 20 µL
- Column Temperature: 30-40°C

Preparation of Solutions

- Blank: Diluent only
- Placebo: Excipients without drug
- Standard: Selpercatinib standard stock diluted to 5 µg/mL
- Sample: Marketed formulation equivalent to 5 µg/mL Selpercatinib

Optimization of Chromatographic Conditions

Various mobile phase compositions were tested (Table 5.23). The best system suitability parameters were achieved with 0.2% TFA and acetonitrile in 50:50 ratios at pH 6.5. The retention time of Selpercatinib was 7.76 ± 0.05 min with sharp, symmetrical peak (Figures 5.10 & 5.11).

Selection of Detection Wavelength

Selpercatinib solution was scanned in the 200–400 nm range. A wavelength of 240 nm was selected based on maximum absorbance.

Selection of Mobile Phase

Based on trial-and-error method a mobile phase of 0.2% trifluoroacetic acid (TFA) and acetonitrile in a 50:50 v/v ratio, with the TFA solution adjusted to pH 6.5 was selected for optimal peak performance.

Preparation of Mobile Phase

To make 100 mL of the 0.2% v/v TFA solution, carefully measure 0.2 ml of trifluoroacetic acid (TFA) using a graduated pipette. Add the TFA to a 100 ml volumetric flask containing approximately 70 ml of HPLC-grade water. Add slowly a solution of sodium hydroxide, adjust the pH 6.5 and add HPLC-grade water to the mark up to the mark. Combine the pH-adjusted 0.2% TFA solution combine with acetonitrile to get the final 50:50 v/v ratio. The final mobile phase was filtered through Whatman No. 41 filter paper.

Preparation of Standard Stock and Working Solutions

Weigh accurately 100 mg of selpercatinib dissolved in acetonitrile and made up volume up to 100 ml. with HPLC grade water to get 1 mg/ml solution (1000 ppm). The working standards of different concentrations were prepared by dilution with HPLC-grade water

Analysis of Marketed Formulation

Selpercatinib powder equivalent to 100 mg was processed as above, filtered, and diluted to obtain the required concentration. The assay was performed by comparing the peak area of sample with standard.

Specificity

The peak response was due to Selpercatinib only, making this method a very specific for this drug.

Specificity is the ability to measure the analyte response in the presence of its potential impurities, degradation products, and matrix components. This study aims to demonstrate the specificity of the RP-HPLC method for Selpercatinib by analyzing blank, placebo, standard, sample, and stressed samples. (Table 1)

The results indicate that the method is specific for Selpercatinib, as there were no interfering peaks at the retention time of the analyte in blank, placebo, or formulation samples. Additionally, the peak was well resolved from degradation products under stress conditions.

Linearity and Range

The linearity of system was found a linear between the concentration ranges of 2-10µg/ml. The correlation

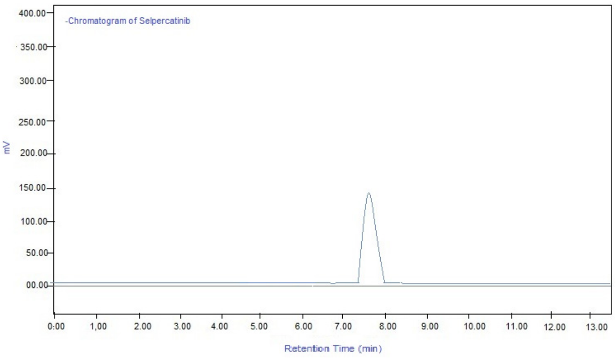

Fig. 1: Chromatogram of Selpercatinib Standard

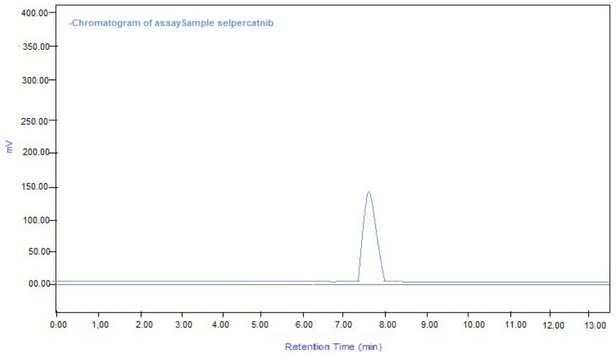

Fig. 2: Chromatogram of Assay

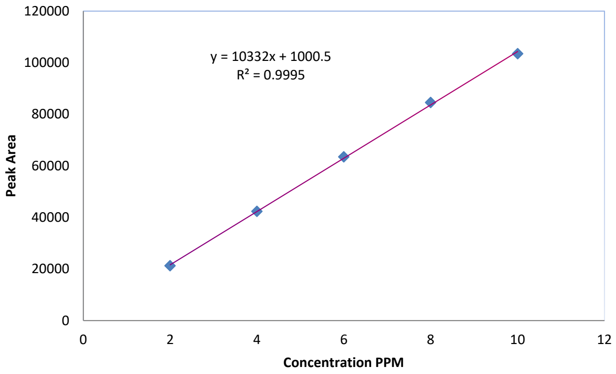

Fig. 3: Calibration curve of Selpercatinib

Table 1: Specificity

Sample type	*Retention time (min)*	*Interfering peaks*	*Peak purity Pass/Fail*
Blank	—	None	—
Placebo	—	None	—
Standard	7.76	No	Pass
Sample	7.76	No	Pass

Table 2: Linearity data of Selpercatinib

S. No.	*Actual concentration PPM*	*Peak area*	*Concentration found PPM*
1	2	21222	1.96
2	4	42332	4.00
3	6	63422	6.04
4	8	84543	8.09
5	10	103433	9.92

Table 3: Regression analysis of Selpercatinib

Regression parameters	*Values*
Wavelength Maxima	240
Regression Equation	10332x + 1000.5
Correlation coefficient(r^2)	0.9995
LOL(µg/ml)	2-10
LOD(µg/ml)	0.257
LOQ(µg/ml)	0.781
Standard deviation	807
Slope	10332

Table 4: Accuracy study of Selpercatinib

Parameters	*Accuracy level*			*Mean % Recovery*
	80%	*100%*	*120%*	
Amount present (µg/ml)	4	4	4	100.61
Amount added (µg/ml)	3.2	4	4.8	
Amount recovered (µg/ml) *	7.22	8.08	8.84	
% Recovery	100.33	101.05	100.45	

*Average triplicate readings

Table 5: Repeatability study of Selpercatinib

S. No.	*Actual concentration µg/ml*	*Peak area*	*Concentration found µg/ml*
1	6	63444	6.04
2	6	63564	6.06
3	6	63445	6.04
4	6	63423	6.04
5	6	63456	6.05
6	6	63234	6.02
		Mean	6.042
		STDV	0.010
		%RSD	0.172

coefficient (r^2) was found to be 0.9995 for Selpercatinib which are near to 0.999, ensuring that a good correlation existed between the peak area and analyte concentration as shown in Figs. 4, 5 and Table 2, & 3.

Further LOD and LOQ concentrations were verified by injecting the six individual standard solutions at these concentration levels.

Accuracy

The mean recovery of Selpercatinib was between 100.61 % was within the accepted criteria (98-102%), which indicates accuracy of the method. The results for the accuracy were shown in table 4.

Precision

Repeatability

The repeatability study which was conducted on the solution having the concentration of about 6 µg/ml showed %RSD was found to be 1.72 for Selpercatinib . It was concluded that the analytical technique showed good repeatability. The data are shown in table 5

Table 6: Intraday precision of Selpercatinib

S. No.	*Analysit I*			*Analysit II*		
	2 µg/ml	*4µg/ml*	*6µg/ml*	*2 µg/ml*	*4µg/ml*	*6µg/ml*
1	21233	42333	63444	23222	43524	64355
2	21233	42343	63564	23552	43255	64535
3	21234	42343	63445	23333	43525	64535
4	21322	42312	63423	23112	43552	64553
5	21232	42113	63456	23211	43578	65443
SD	39.81	99.09	55.85	168.05	131.48	431.82
MEAN	21250.8	42288.8	63466.4	23286	43486.8	64684.2
%RSD	0.187	0.234	0.088	0.722	0.302	0.668
Mean % RSD	0.170			0.564		

Table 7: Inter day precision of Selpercatinib

	S. No.								
	Con	*1*	*2*	*3*	*4*	*5*	*SD*	*Mean*	*%RSD*
Day 1	2	21222	21333	21232	21343	21221	62.14	21270.2	0.292
	4	42332	42332	42123	42332	42355	96.55	42294.8	0.228
	6	63422	63552	63552	63552	63552	58.14	63526	0.092
Day 2	2	21383	21343	21222	21343	21366	63.44	21331.4	0.297
	4	42666	42322	42332	42356	42366	145.09	42408.4	0.342
	6	63552	63455	63422	63425	63442	53.57	63459.2	0.084
Day 3	2	21263	21334	21343	21453	21222	88.35	21323	0.414
	4	42535	42536	42353	42543	42543	83.38	42502	0.196
	6	63255	63454	63554	63454	63422	108.62	63427.8	0.171
Mean %RSD				0.235					

Table 8: Robustness study of Selpercatinib

Parameter	*Sample*	*1*	*2*	*3*	*Mean*	*SD*	*%RSD*
Flow rate ml./min	Flow Rate: 0.9 ml/min (Decreased by 10%)	42332	42533	42334	42399.7	115.47	0.272
	Flow Rate: 1.0 ml/min	43244	43244	43234	43443	5.77	0.013
	Flow Rate: 1.1 ml/min (Increased by 10%)	44334	44322	44332	44329.3	6.43	0.015
Wavelength nm	245	44343	43333	43332	43669.3	476.35	1.091
	246	44332	44352	44355	44346.3	10.21	0.023
	243	44344	44355	44344	44347.7	5.19	0.012
Colum Tem	Column Temperature: 360 C (Decreased by 10%)	45552	45255	45255	45266	171.47	0.379
	Column Temperature: 400 C	45524	45262	45244	45343.3	156.72	0.346
	Column Temperature: 440 C (Increased by 10%)	45662	45633	45334	45543	181.58	0.399
						Mean % RSD	0.265

Table 9: System suitability parameter of Selpercatinib

S. No.	*Parameters*	*Limit*	*Results*
1	Injection precision	RSD < 1% for n≥5	0.165 % n=5
2	Tailing factor	T ≤ 2	0.45
3	Theoretical plate	N > 2000	7874
4	Retention time	7.76	

Table 10: Assay of Selpercatinib

Parameter	*Value*
Area of Standard	42322
Area of Sample	42456
Standard Concentration (µg/mL)	5
Sample Concentration (µg/mL)	5
Dilution Factor	1
Assay %	99.68
Acceptance Range	95.0% to 105.0%

Intermediate precision

The intra-day and inter-day precision study, which were conducted, showed a %RSD of 0.367% for intraday analysis by different analyst and different concentrations and 0.235% for inter day precision, thus the data showed that the RSD was below 2% inferring that the analytical technique had a good intraday and Interday precision. The data are shown in table 6 and 7.

Robustness

The robustness of the method was studied by making slight changes in the chromatographic conditions. Effect of increase and decrease in Flow Rate, Column Temperature and Detector Temperature was studied. There was no significant change observed with slight changes in chromatographic parameters which demonstrate that the developed method is robust. The results of robustness study are reported in table-8.

System Suitability Parameter

System suitability parameters obtained with 20µl injection volume are summarized in table 532. As it is evident from the system suitability parameters, Selpercatinib get eluted well near ten minutes and their resolution is very satisfactory, thus making it a very economical method for routine analysis of drug. Following system suitability test parameters were established. (Table 9)

Tailing Factor for Selpercatinib Peak from Standard Solution was found less than 2.0. Theoretical Plates for Selpercatinib Peaks from Standard Solution was found more than 2000. % RSD of area count of Selpercatinib from six replicate injections of Standard Solution was found less than 2.0 %. % RSD of assay results was found less than 2.0%.

Estimation of Selpercatinib in dosage form

The retention time of Selpercatinib was 7.76 ± 0.051 min and system suitability criteria was met using the proposed method.

As per the methodology, Blank solution, Standard solution and Sample solutions were injected and system suitability criteria was observed.

Standard Solution

Weigh accurately 10 mg of Selpercatinib reference standard, dissolve in 5 ml methanol and make up to 100 mL (100 mg/ml). Prepare 5 µg/mL working standard by further dilution. Dilute 5 ml up to 100ml with water. Filter through 0.45 µm filter.

Sample Solution

Take an amount of injection equivalent to 10 mg Selpercatinib, dilute with 5 ml methanol to 100 ml, and further dilute to match the concentration of the standard (100 mg/ml).

Prepare 5 µg/mL working standard by further dilution (dilute 5 ml up to 100ml with water). Filter through 0.45 µm membrane filter.

Assay (%) is calculated using the formula:

Assay (%) = (Area_sample / Area_standard) × (Concentration_standard / Concentration_sample) × Dilution Factor × 100

Calculation

Data used in the calculation:

Assay (%) = (42322 /42456) × (5 / 5) × 1 × 100 = 99.68 %

As per pharmacopeial standards, Selpercatinib content should be within 95.0% to 105.0% of the labeled amount shown in table 10.

The reference chromatogram of Selpercatinib Blank, Standard and Sample solutions using the proposed method is shown in Figure 2 and 3 respectively.

Solution Stability:

Solution stability shall be performed on standard & test solution preparation at specified time interval at room temperature and % RSD shall be calculated. The solution stability shall be checked at initial, 3 hr, 6 hr, 12 hr, 18 hr

Table 11: Solution stability

S. No.	*Time*	*At Room temperature*		*At 10^0C*	
		Peak area of standard solution	*% Assay results*	*Peak area of standard solution*	*% Assay results*
1	Initial	103433	100	103453	100.02
2	After 1 Hour	103233	99.81	102333	98.94
3	After 3 Hours	103232	99.81	103233	99.81
4	After 6 Hours	103221	99.80	103244	99.82
5	After 12 Hours	103434	100.00	104322	100.86
6	After 18 Hours	103433	100.00	103422	99.99
7	After 24 Hours	104332	100.87	103433	100.00
	Mean	103474.00	100.04	103348.57	99.92
	STDV	391.96	0.38	581.19	0.56
% RSD		0.379	0.379	0.562	0.562
Acceptance Criteria		NMT 2.0%			

and 24 hr. The results of solution stability study are reported in Table 11.

RESULTS AND DISCUSSION

The optimized RP-HPLC method offers rapid, sensitive, and precise quantitation of Selpercatinib with high specificity and robustness. The method complies fully with ICH guidelines and is suitable for routine quality control. Compared to existing literature [1-4], the method is economical with short runtime and reliable accuracy and precision metrics.

CONCLUSION

A validated RP-HPLC method was successfully developed for estimation of Selpercatinib in bulk and pharmaceutical dosage forms. The method is simple, precise, accurate, robust, and suitable for routine analysis in quality control labs.

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