EXPLORING THE DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR SELPERCATINIB ANALYSIS IN HUMAN PLASMA

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***Abstract*- A rapid fast, accurate, precise method was developed for estimation of selpercatinib in human plasma using darunavir as an internal standard by reversephase high perfomence liquid chromatography (RP-HPLC). The separation was carried out by Kromosil C_{18} (250mm x 4.6 mm, 5µ), Mobile phase disodium hydrogen phosphate and methanol (55:45)V/V. The analysis performed at flow rate was maintained as 1.0 ml/min, using PDA detector wavelength at 218 nm. Column temperature was set and maintained at 30^0C Injection volume 10µL Run time 5.0 min for better separation and resolution. The retention time of selpercatinib was found to be 2.334mins. The recovery for selpercatinib was found to be 99.523%. The method was found to be linear between the concentration range of 150 to 6000 ng/ml (r^2= 0.999). the lower limits of quantification were 150 ng/ml, which drug reach the blood plasma at the level of 150 ng/ml. The reported method validated as per ICH guidelines and found to be in a suitable range.**

***Keywords*: Selpercatinib, Darunavir, RP-HPLC, Human plasma, Bioanalytical, Method development, Validation.**

INTRODUCTION

Selpercatinib is an oral anti-cancer drugs it is mainly used for the treatment if non small cell lung cancer (NSCLC) [1] and also used for the treatment of thyroid cancer. It comes under the classification of tyrosine kinase inhibitor which is {IUPAC name}[2]. Selpercatinib is approved in the year of 2020 [3]. A literature review reveals that very few analytical methods have been reported for the determination of selpercatinib using UPLC, hyphenated techniques. However, a literature review reveals that no method reported for determination of selpercatinib using RP-HPLC. Hence a precise, sensitive, accurate, selective, reproducible, and rapid analytical technique for the estimation of selpercatinib in human plasma is developed and validated as per ICH guidelines. The chemical structure of selpercatinibis shown in **Fig.1**

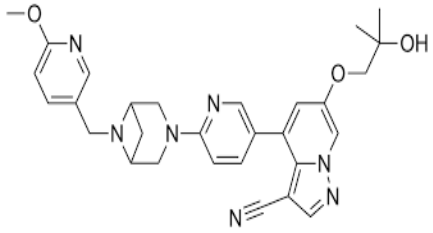

Fig.1: CHEMICAL STRUCTURE OF SELPERCATINIB

The proposed method is simple and economical bioanalytical method for determination of serlpercatinib in spiked human plasma using darunavir as an internal standard (IS). Internal standard satisfy with various analytical errors in methods by improving accuracy, precision and robustness of bioanalytical method.

MATERIALS AND METHODS

Reagent and chemicals: The drug sample of serlpercatinib Jai Ram Biosciences, Kukatpally, Hyderabad, Internal Standard darunavir from Akrivis Pharma pvt Ltd.
The K_2 EDTA controlled Blood plasma obtained from Deccan Pathological labs, Hyderabad
Analytical graded Water, Acetonitrile, Phosphate buffer, Methanol, Sodium dihydrogen phosphate, Ortho-phosphoric acid from Rankem, Avantor performance material India limited.
Instrumentation and chromatographic conditions: The ElectronicsBalance-Denver, p H meter -BVK enterprises, India, Sonicator -BVK enterprises, Centrifuge -Thermo Fisher, Vertex -Remi CM101, WATERS HPLC 2695

SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software, column Kromosil C_{18} (250mm x 4.6 mm, 5µ), Mobile phase disodium hydrogen phosphate and methanol (55:45)V/V and Flow rate was maintained as 1.0 ml/min, using Detector wavelength was 218 nm.

Preparation of the solution:

Diluent: Based up on the solubility of the drugs, diluent was selected, 0.01N Potassium dihydrogen phosphate and acetonitrile taken in the ratio of 55:45.

Extraction procedure

Take 750µl of plasma and 0.5µl of internal standard, 0.25µl of Selpercatinib from the spiking solutions of both into a centrifuging tube and add 1 ml of Acetonitrile go for cyclomixer for 15 sec. Then vertex for 2 min and finally centrifuge for 5 min at 3200 rpm speed. After the centrifugation collect the sample and filter it directly inject 10 µL into HPLC.

750µl of plasma +500µl of internal standard, +250µl of Selpercatinib
|
15 sec cyclomixer
|
1 ml of acetonitrile
|
Vertex for 2 min
|
Centrifuge for 5 min at 3200 rpm
|
Collection of supernatant sample
|
Filter the sample (polyvinylidene fluoride or polyvinylidene difluoride 0.45µ filter)
|
Inject 10 µL into HPLC

Preparation of Selpercatinib Spiking Solutions:

From the above Selpercatinib stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluents to produce 0.150ug/ml, 0.300ug/ml, 0.450ug/ml, 1.200ug/ml, 3.0ug/ml, 3.6ug/ml,4.8ug/ml and 6.0ug/ml. Quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 150ng/ml, 300ng/ml, 450ng/ml, 1200ng/ml, 3000ng/ml, 3600ng/ml, 4800 ng/ml, 6000 ng/ml.

Final concentration: From the above solution, take 0.5ml of solution and spiking blank plasma with working stock dilutions of analyte to produce 10ug/ml ISD concentration.

RESULT AND DISCUSSION

Method development: Chromatographic conditions used was Column Kromosil C_{18} (250mm x 4.6 mm, 5µ), Mobile phase disodium hydrogen phosphate and methanol (80:20)V/V and Flow rate was maintained as 1.0 ml/min, using Detector wavelength was 218 nm and Column temperature was set to 30^0C Injection volume 10µL Run time 5.0 min. The retention time of selpercatinib and darunavir was found to be 2.334mins and 2.953mins respectively. The chromatogram of selpercatinib obtained by optimized condition is shown in **Fig.2**

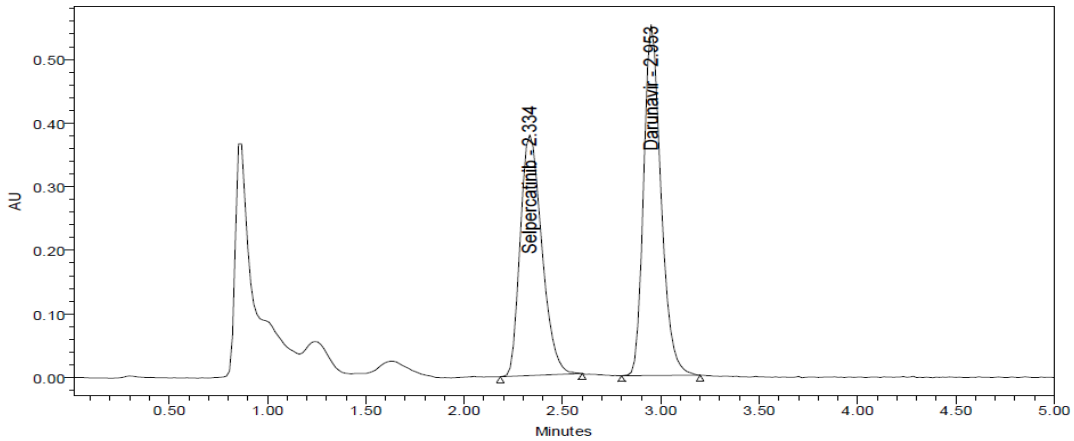

Fig .2: OPTMIZED CHROMATOGRAM

Bioanalytical Method Validation: The developed bioanalytical method was validated as per ICH guidelines.

System suitability of Selpercatinib

plate count, tailing factor, resolution of Selpercatinib was According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The % CV of the retention time (RT) should be ≤ 2.00 %. The result is summarized in **Table 1.**

Table 1: System Suitability of Selpercatinib

s.no	RT	Area	USP Plate Count	USP Tailing	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	2.328	1124626	2287	1.25	2.959	1973811	5285	1.23	3.4
2	2.328	1124904	2284	1.26	2.961	1971649	5312	1.22	3.5
3	2.330	1139916	2287	1.27	2.962	1972318	5267	1.21	3.5
4	2.345	1112020	2388	1.24	2.972	1974412	5129	1.26	3.4
5	2.345	1122813	2349	1.27	2.978	1893738	5256	1.23	3.5
6	2.347	1140576	2335	1.25	2.978	1952477	5292	1.21	3.5
Mean	2.337	1127476			2.968	1956401			
Std. Dev.		10970.3				31797.5			
% RSD		1.0				1.6			

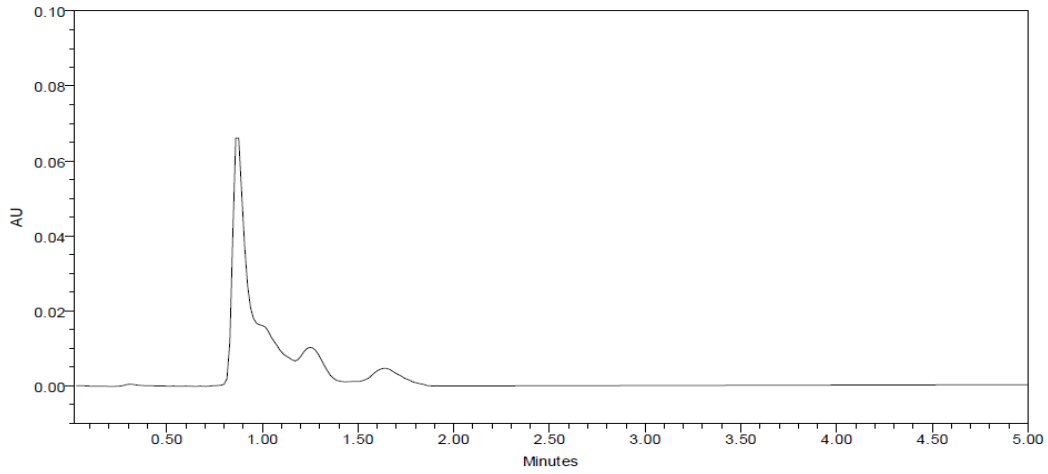

Fig. 2: Chromatogram of a Blank Plasma Sample

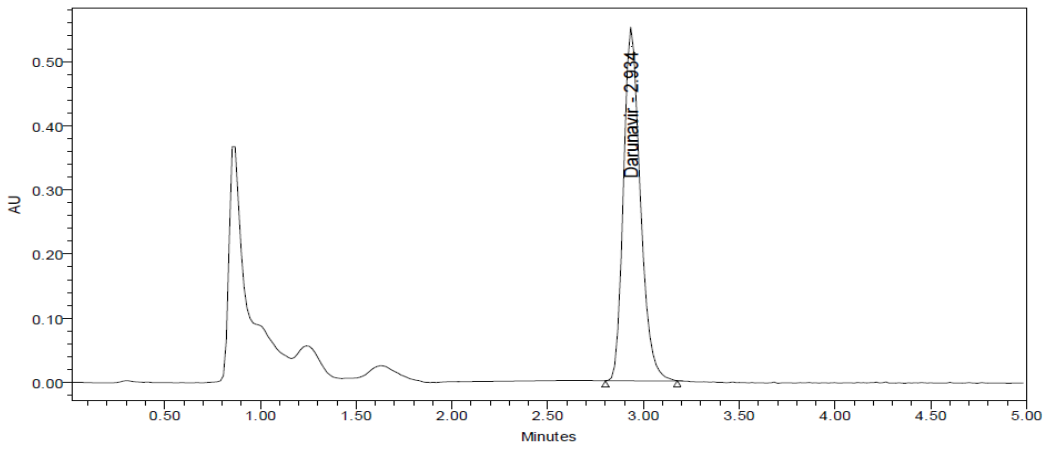

Fig. 3: Representative Chromatogram of Blank Plasma with Internal Standard

Linearity:
Calibration was found to be linear over the concentration range of 150 to 6000ng/ml. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analyte.

Table 2: Linearity of Selpercatinib

S.NO	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Nominal Concentration (ng/ml)							
	150.000	300.000	450.000	1200.000	3000.000	3600.000	4800.000	6000.000
	Nominal Concentration Range (ng/ml)							
	(120.000-180.000)	(255.000-345.000)	(382.500-517.500)	(1,020.000-1,380.000)	(2,550.000-3,450.000)	(3,060.000-4,140.000)	(4,080.000-5,520.000)	(5,100.000-6,900.000)
	Back Calculated Concentration (ng/ml)							
1	147.895	298.745	449.895	1199.458	2999.758	3589.784	4769.785	5989.745
2	149.854	296.789	447.856	1189.785	2994.485	3598.745	4789.586	5978.987
3	150.415	301.120	451.102	1201.524	3001.120	3601.458	4801.598	5999.785
Mean	149.3880	298.8847	449.6177	1196.9223	2998.4543	3596.6623	4786.9897	5989.5057
SD	1.32305	2.16888	1.64067	6.26684	3.50435	6.10931	16.06463	10.40107
%CV	0.89	0.73	0.36	0.52	0.12	0.17	0.34	0.17
% Mean Accuracy	99.59	99.63	99.92	99.74	99.95	99.91	99.73	99.83

Table 3: linearity of ISD and drug

Final concentration (µg/ml)	**ISD (area)**	**Drug (area)**	**Area response ratio**
0	0	0	0
150	1968541	54211	0.028
300	1963257	102285	0.052
450	1982745	164562	0.083
1200	1986452	432858	0.218
3000	1985455	1078635	0.543
3600	1984562	1308658	0.659
4800	1985625	1689652	0.851
6000	1987458	2165895	1.090

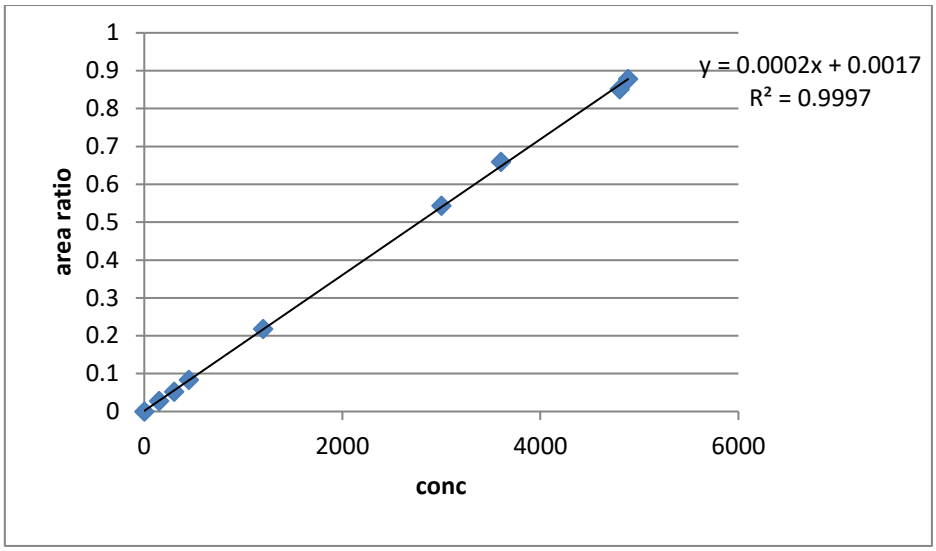

Fig. 4: Calibration Curve for Regression Analysis

Precision and accuracy (intra-day runs of Selpercatinib)

The intraday and interday accuracy and precision were assessed by analyzing six replicates at five different QC levels such as LLOQ, LQC, MQC and HQC. Accuracy and precision method performance was evaluated by determined six replicates analysis for selpercatinib at four concentration level, i.e., 450ng/ml (LQC) 3000 ng/ml (MQC), 4800 ng/ml (HQC), 150 ng/ml (LLOQ).

Table 4: precision data for intra-day runs of Selpercatinib

S. No.	HQC	MQC1	LQC	LLOQ QC
	Nominal Concentration (ng/ml)			
	4800.000	3000.000	450.000	150.000
	Nominal Concentration Range (ng/ml)			
	(4,080.000-5,520.000)	(2,550.000-3,450.000)	(382.500-517.500)	(120.000-180.000)
	Back Calculated Concentration (ng/ml)			
1	4796.785	2998.874	449.870	147.895
2	4785.987	2989.785	447.880	149.258
3	4782.785	2987.432	444.789	146.986
4	4801.120	2974.897	450.897	151.785
5	4769.895	3001.784	441.895	148.785
6	4779.510	2989.896	442.842	146.869
N	6	6	6	6
Mean	4786.0137	2990.4447	446.3621	148.5963

SD	11.46086	9.49923	3.74149	1.82810
%CV	0.24	0.32	0.84	1.23
% Mean Accuracy	99.71	99.68	99.19	99.06
1	4793.785	2994.785	448.598	147.895
2	4789.585	2996.210	450.365	148.874
3	4778.965	2987.432	444.789	146.986
4	4801.120	2974.897	449.652	152.450
5	4756.147	2998.452	441.542	148.785
6	4769.896	2989.896	443.874	147.854
N	6	6	6	6
Mean	4781.5830	2990.2787	446.4700	148.8073
SD	16.64361	8.56898	3.56860	1.91481
%CV	0.35	0.29	0.80	1.29
% Mean Accuracy	99.62	99.68	99.22	99.20
1	4791.895	2997.852	385.320	146.895
2	4778.685	2969.652	396.124	149.842
3	4776.521	2987.365	449.234	146.986
4	4789.452	2974.897	460.254	151.783
5	4756.147	2993.452	498.120	148.785
6	4795.821	3001.250	510.101	146.698
N	6	6	6	6
Mean	4781.4202	2987.4113	449.8588	148.4982
SD	14.50402	12.72015	51.22383	2.03842
%CV	0.30	0.43	11.39	1.37
% Mean Accuracy	99.61	99.58	99.97	99.00
Between Batch Precision and Accuracy				
N	18	18	18	18

Mean	4783.0056	2989.3782	447.5636	148.6339
SD	13.66652	9.88827	27.97112	1.81687
%CV	0.29	0.33	6.25	1.22
% Mean Accuracy	99.65	99.65	99.46	99.09

The intraday and interday accuracy of plasma samples were assessed and excellent mean %accuracy was obtained with range varied from 99.00% - 99.66% for interday and 99.10% - 101.90% for interday respectively. The precision (%CV) of the analyte the plasma sample were calculated and found to be 0.25% -11.45 %_ for intraday and 0.35% - 0.85%_ for interday, respectively. The results are summarized in **Table 4,5.**

Table 5: precision data for inter-day runs of Selpercatinib

S.No	HQC	MQC1	LQC	LLOQ QC
	Nominal Concentration (ng/ml)			
	4800.000	3000.000	450.000	150.000
	Nominal Concentration Range (ng/ml)			
	(4,080.000-5,520.000)	(2,550.000-3,450.000)	(382.500-517.500)	(120.000-180.000)
	Calculated Concentration (ng/ml)			
1	4785.758	2994.874	448.863	149.752
2	4765.895	2992.560	449.745	145.631
3	4778.965	2963.752	445.236	148.962
4	4801.120	2974.897	450.897	150.120
5	4756.147	3001.784	441.895	148.989
6	4769.896	2989.896	442.842	147.874
N	6	6	6	6
Mean	4776.2968	2986.2938	446.5797	148.5547
SD	15.92487	14.16917	3.78402	1.62786
% CV	0.33	0.47	0.85	1.10
% Mean Accuracy	99.51	99.54	99.24	99.04
1	4788.526	2990.452	449.875	145.687
2	4775.698	2996.420	447.852	146.752
3	4785.240	2963.752	444.000	149.750
4	4801.120	2974.897	448.893	151.120
5	4755.360	3002.475	441.895	148.963
6	4764.256	2985.452	443.852	149.896
N	6	6	6	6
Mean	4778.3667	2985.5747	446.0612	148.6947
SD	16.75535	14.26385	3.23288	2.06549
% CV	0.35	0.48	0.72	1.39
% Mean Accuracy	99.55	99.52	99.12	99.13

RECOVERY

Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing same area with a known amount of selpercatinib the over all %mean recovery for selpercatinib was found to be **99.523%**.

The recovery obtained for selpercatinib at three QC concentrations levels are summarized in **Table 6_** respectively. The overall % mean recovery darunavir was found be **99.43%** the recovery result darunavir (ISD) summarized in **Table 6- Recovery of Selpercatinib**

Table 6: Recovery of Selpercatinib

S.No	**HQC**		**MQC1**		**LQC**	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	147733	144077	91409	90396	13921	13869
2	148405	148841	91176	90379	13845	13804
3	143539	142713	91727	90872	13758	13870
4	147115	147096	91322	90120	13868	13756
5	145983	142915	90972	90745	14037	13983
6	142321	146531	90871	90208	14040	13997
N	6	6	6	6	6	6
Mean	145849	145362	91246	90453	13912	13880
SD	2428.42	2495.32	311.23	296.80	111.56	95.57
% CV	**1.67**	**1.72**	**0.34**	**0.33**	**0.80**	**0.69**
% Mean Recovery	99.67		99.13		99.77	
Overall % Mean Recovery	99.523					
Overall SD	0.3437					
Overall % CV	**0.35**					

Recovery - Internal standard

Table 7: Recovery of Darunavir (IS)

S.No.	Un extracted Area Ratio	Extracted Area Ratio
1	556372	554111
2	559636	550373
3	556452	555743
4	559592	552130
5	558084	557544
6	554347	555372
N	6	6
Mean	557413.8	554212.2
SD	2075.94	2602.87
% CV	0.37	0.47
% Mean Recovery	99.43	

Stabilities

In bench-top stability, six replicates of LQC & HQC samples (37.5 and 400ng/ml) were analyzed for 9 hours at room temperature on the laboratory bench. The % mean stability was calculated and found to 99.42% for LQC and 99.05% for HQC respectively.

Matrix Sample stability at -28 ± 5 and -80 ± 5 ℃ for 37 days:

Long term stock solution stability for the selpercatinib was determined at a concentration of LQC- HQC level after a storage period of 37 days at -28℃ and -80℃ in the refrigerator.99.54, 99.70, 99.30 and 99.12 at -28 ± 5 ℃ and 99.66, 99.69, 99.05 and 99.56 at -80 ± 5 ℃ separately. The long term stability of selpercatinib presented in **Table 8,9,10**

Long term stock solution stability

Table 8: stability of Selpercatinib (zero days)

S.No.	**HQC**	**LQC**
	Nominal Concentration (ng/ml)	
	4800.000	450.000
	Nominal Concentration Range (ng/ml)	
	(4,080.000-5,520.000)	(382.500-517.500)
	Calculated Concentration (ng/ml)	
1	4795.254	448.365
2	4775.632	449.785
3	4763.452	446.698
4	4779.365	445.632
5	4759.890	442.523
6	4759.368	439.986
N	6	6
Mean	4772.1602	445.4982
SD	14.05217	3.66848
% CV	**0.29**	**0.82**
% Mean Accuracy	**99.42**	**99.05**

Matrix samples stability at -28±5 °C for 37 days

Table 9: Matrix samples stability at -28±5 °C for 37 days

S.No.	HQC		LQC	
	Nominal Concentration (ng/ml)			
	4800.000	4800.000	450.000	450.000
	Nominal Concentration Range (ng/ml)			
	(4,080.000-5,520.000)	(4,080.000-5,520.000)	(382.500-517.500)	(382.500-517.500)
	Calculated Concentration (ng/ml)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	4788.526	4791.523	448.863	449.852
2	4774.520	4785.631	449.745	446.364
3	4783.142	4769.895	446.362	448.632
4	4801.120	4799.365	450.897	442.365
5	4756.140	4802.320	444.201	441.895
6	4764.256	4763.562	441.025	447.254
N	6	6	6	6
Mean	4777.9507	4785.3827	446.8488	446.0603
SD	16.43547	15.72357	3.74159	3.27148
% CV	0.34	0.33	0.84	0.73
%Mean	99.54	99.70	99.30	99.12

Accuracy				
% Mean Stability	100.16		99.82	

Matrix samples stability at -80±5 °C for 37days

Table 10: Matrix samples stability at -80±5 °C for 37 days

S.No.	HQC		LQC	
	Nominal Concentration (ng/ml)			
	4800.000	4800.000	450.000	450.000
	Nominal Concentration Range (ng/ml)			
	(4,080.000-5,520.000)	(4,080.000-5,520.000)	(382.500-517.500)	(382.500-517.500)
	Calculated Concentration (ng/ml)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	4793.785	4782.652	449.870	448.863
2	4785.365	4775.360	446.520	449.745
3	4796.230	4789.230	442.250	445.236
4	4801.120	4798.302	450.897	447.210
5	4756.147	4763.854	441.895	446.130
6	4769.896	4801.230	442.842	451.021
N	6	6	6	6
Mean	4783.7572	4785.1047	445.7123	448.0342
SD	17.40480	14.16883	3.99044	2.22126
% CV	0.36	0.30	0.90	0.50
%Mean Accuracy	99.66	99.69	99.05	99.56
% Mean Stability	100.03		100.52	

Conclusion:

A simple, accurate, precise method was developed for the estimation of the Selpercatinib in Blood plasma using the Darunavir as internal standard. Retention time of Selpercatinib was found to be 2.334min. and IS was found to be 2.953min, which reach the level of both drugs possibly found in Blood plasma. Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range. The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories

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