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Analytical Method Development and Validation for Determination of Selpercatinib by Using RP-HPLC

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ABSTRACT

A novel, specific and precise RP-HPLC method has been developed and validated for the quantification of selpercatinib in pure and its pharmaceutical dosage form. Selpercatinib is a kinase interrupter with increased selectivity for rearranged during transfection (RET) tyrosine kinase receptors (RTKs) above the additional RTK classes. It is used for the treatment of RET fusion-positive non-small cell lung carcinoma (NSCLC). The segregation was accomplished on the Zorbax C18 column (150 x 4.6 mm) with a 5 μ particle size. 0.1% orthophosphoric acid and Acetonitrile (ACN) (60:40 v/v) was used as an optimized mobile phase at a flow rate of 1ml/min. The wavelength selected was 220.0nm. The retention time for selpercatinib was 2.653 min. The linearity of selpercatinib was detected to be 5- 30μg/ml. Linearity equations obtained for selpercatinib was y = 18428x + 2196.2 with correlation coefficient 0.999. The % RSD for precision was found to be less than 2%. %Recovery was obtained as 99.74% for selpercatinib. The LOD and LOQ for selpercatinib were obtained as 0.02 μg/ml and 0.05 μg/ml.

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INTRODUCTION

Selpercatinib ($C_{29}H_{31}N_7O_3$) is chemically 6-(2-hydroxy-2-methyl propoxy)-4-[6-[6-[(6-methoxy pyridin-3-yl)methyl]-3,6-diazabicyclo [3.1.1] heptan-3-yl]pyridin-3-yl] pyrazolo [1,5-a] pyridine-3-carbonitrile. The molecular weight of selpercatinib is 525.6 g/mol. It is a kinase inhibitor with improved selectivity for rearranged during transfection (RET) tyrosine kinase receptors (RTKs) above the additional RTK classes. Selpercatinib is indicated to treat non-small cell lung carcinoma (Goto *et al.*, 2020) and 2 types of thyroid cancers with alterations in the RET gene together with advanced medullary thyroid cancer (Anthony, 2020).

Selpercatinib (Retevmo) is an orally bioavailable wild-type selective inhibitor, fusion, and mutant products concerning the proto-oncogene receptor tyrosine kinase RET, with possible antineoplastic activity. When administered orally, it specifically binds to and targets different RET-containing fusion products and RET mutants which results in cell growth inhibition of tumor cells that show enhanced RET activity.

The chemical structure of selpercatinib was shown in Figure 1. A literature survey on selpercatinib revealed that LC-MS (Wang *et al.*, 2020) and LC-MS/MS (Şenturk *et al.*, 2020) methods were reported so far for the determination of selpercatinib. The aim of the study is to establish novel, specific and precise reverse-phase high-performance liquid chromatography method for the quantifica-

tion of selpercatinib in pure and its capsule dosage form.

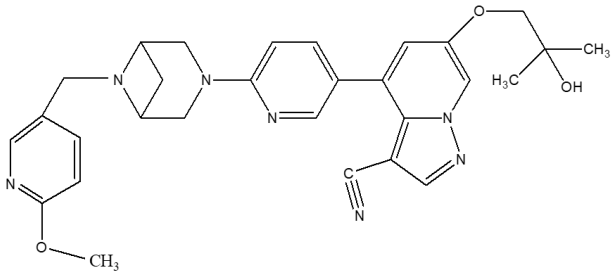

Figure 1: Structure of Selpercatinib

MATERIALS AND METHODS

Instruments

Waters 2695 HPLC system equipped with quaternary pumps and photodiode array (PDA) detector was utilised. The pH of the solutions was calculated by a pH meter (BVK enterprises, India). All analytical measurements were done on Analytical balance (Denver).

Chemicals and reagents

Selpercatinib sample was obtained as a gift sample from Spectrum Pharma Research Solutions (Hyderabad). Ortho-phosphoric acid, methanol, potassium dihydrogen orthophosphate, acetonitrile were purchased from Rankem Laboratories Pvt Ltd. Millipore Milli Q water was prepared in the laboratory.

Preparation of solutions

Preparation of diluents

Acetonitrile and water in a ratio of 50:50 v/v was utilised as a diluent

Preparation of standard stock solutions

10mg of selpercatinib was weighed precisely and taken into a 50ml volumetric flask. The volume was then made up with diluent to obtain a drug standard stock solution of 200μg/ml concentration.

Preparation of standard working solutions

Drug standard stock solution (1ml) was transferred to five individual volumetric flasks (10ml). The volume was then made up with diluent to obtain a drug concentration ranging from 5μg/ml-30μg/ml.

Preparation of sample stock solutions

Ten capsules were weighed, the average weight of each capsule was determined. The weight identical to one capsule was then taken into a volumetric flask (100ml) and diluent (50 ml) was transferred to the flask. For 25 min flask was sonicated. The volume was then made up to the mark and filtered by using HPLC filters (200μg/ml of selpercatinib).

Preparation of sample working solutions

Filtered sample stock solution (0.5 ml) was taken into a volumetric flask (10ml) and made up to volume with diluent (20μg/ml of selpercatinib).

Method Validation

System suitability parameters

System suitability was considered under every validation parameter by introducing six replicate injections of the drug standard solution (20μg/ml). Parameters like tailing factor, plate count and column efficiency were noted (Latha *et al.*, 2017).

Specificity

The specificity was assessed by comparing the placebo solution with the drug solution by introducing the samples into the HPLC system. The resulting chromatograms were seen for the interference of placebo response with a drug peak response (AnjaneyuluReddy *et al.*, 2019).

Linearity

Linearity is the method capability to assemble test results that are proportionate to the concentration of an analyte in samples within a specified range. Dissimilar drug standard solutions were made to evaluate the linearity by diluting the drug stock solution with the diluents in different concentrations of selpercatinib ranging from 5 μg/ml to 30 μg/ml. The linearity plot of the calibration curve was assessed by linear regression analysis (Manikandan and Lakshmi, 2012).

Limit of detection

The LOD is the smallest concentration of the analyte in a sample that can be detected but not determined. The LOD was determined by using the following formula (Adison, F and Sanjay Pai, P. N. , 2019).

Limit of detection= 3.3 X Standard deviation of the response /slope of calibration curve of the analyte.

Limit of quantification

The LOQ is the smallest concentration of an analyte in a sample, which might be quantified with appropriate accuracy and precision. The LOQ was determined by using the following formula.

Limit of quantification = 10 X Standard deviation of the response /slope of calibration curve of the analyte.

Accuracy

Accuracy is the degree of closeness of test results to the accurate value. Accuracy was performed at 50%, 100% and 150% by adding an acknowledged amount of sample stock solution of selpercatinib (0.5,1,1.5 μg/ml) to the standard stock solution. The

percentage of recoveries were calculated (Diksha *et al.*, 2013).

Precision

Precision is the degree of closeness between the detector responses acquired by several individual estimations of the same sample under specified conditions. Precision was determined by introducing six replicates of selpercatinib standard solution into the HPLC system and % RSD was calculated (Chinmaya *et al.*, 2018).

Robustness

Robustness is an estimate of its capability to remain unchanged by little, but intentional changes in parameters of the analytical method and gives a suggestion of its consistency throughout usage. It was performed by varying the flow rate, temperature, ratio of the mobile phase (Raja *et al.*, 2015).

Forced degradation studies

Oxidation

One ml of 20% hydrogen peroxide (H_2O_2) was added to one ml of selpercatinib stock solution**.** The solution was heated at 60ºC for 30 minutes on a water bath. Then the solution was cooled and made up to the mark using diluent. 10μl of this solution was introduced into HPLC the system (Bhanu *et al.*, 2020).

Acid degradation

One ml of 2N hydrochloric acid (HCl) was added to a drug stock solution. For 30 minutes, the solution was heated at 60ºC on a water bath. Then the solution was cooled and 1 ml 2N sodium hydroxide was added. The solution was made up to the mark using diluent. 10μl of the solution was introduced into the system.

Alkali degradation

One ml of 2N sodium hydroxide (NaOH) was added to the drug solution. The solution was heated for 30 minutes at 60ºC on a water bath. Then the solution was cooled and 1 ml 2N HCl was added. The solution made up with diluent. Ten μl of this solution was introduced into the system.

Dry heat degradation

The drug standard solution was kept in the oven for 6hrs at 105°C. The solution was made up with diluent. 10μl of the solution was introduced into the system.

Photolytic degradation

200μg/ml selpercatinib solution was revealed to UV light by placing a beaker in UV Chamber for 200 Watt-hours/m^2or 7 days in a photostability chamber. Then the solution was diluted and made up to volume with the diluent. 10μl of this solution was introduced into the HPLC system (Mathrusri *et al.*, 2019).

Neutral degradation

Neutral degradation was determined by refluxing the drug in the water at 60ºC temperature for 6hrs. The solution was made up to volume with the diluent. 10μl of this solution was introduced into the HPLC system.

RESULTS AND DISCUSSION

Novel RP HPLC validated technique has been established for the quantification of selpercatinib. A Zorbax C18 column (150 x 4.6 mm, i.d. 5μm particle size) was selected with mobile phase composition 0.1% orthophosphoric acid:ACN (60:40 v/v). The flow rate was kept at 1.0ml/min for the determination of selpercatinib. The developed chromatographic conditions were tabulated in Table 1. The results for all the parameters were summarised in Table 12.

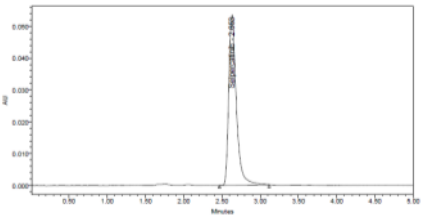

Figure 2: Optimized chromatogram of Selpercatinib

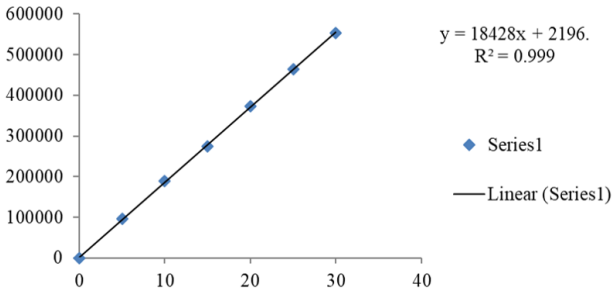

Figure 3: Calibration curve of Selpercatinib

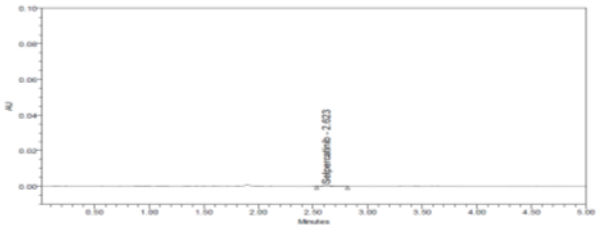

Figure 4: LOD of Selpercatinib

System suitability parameters

A system suitability test was an essential part of the method development to confirm that the system is

Table 1: Optimized chromatographic conditions

Parameter	Chromatographic conditions
Stationary Phase	ZorbaxC18 150x4.6 mm, 5μ
Mobile Phase	0.1% OPA: acetonitrile (60:40 v/v)
Column temperature	30°C
Injection Volume	10 μL
Total Run Time	6 min
Detector	Photodiode array detector
Elution	Isocratic mode
Flow Rate	1.0ml/min
λ_{max}	220 nm

Table 2: System suitability data

Parameter	Selpercatinib
Retention time	2.653
Theoretical plates	3712
Tailing factor	1.47

Table 3: Linearity data of selpercatinib

Linearity level (%)	Conc. (μg/ml)	Peak area
25	5	95634
50	10	189370
75	15	274716
100	20	373485
125	25	464247
150	30	552859

Table 4: Accuracy data of selpercatinib

% Level	Amount Spiked (μg/ml)	Amount recovered (μg/ml)	% Recovery	Mean
50%	10	9.98	99.78	99.74%
	10	10.09	100.87	
	10	9.96	99.59	
100%	20	19.72	98.61	
	20	20.03	100.15	
	20	19.98	99.89	
150%	30	29.83	99.42	
	30	29.72	99.05	
	30	30.09	100.31	

*Mean of three replicates

Table 5: System precision data of selpercatinib

S. No.	Area of selpercatinib
1	369172
2	368855
3	366301
4	370186
5	372102
6	366276
Mean	368815
S.D	2261.8
% RSD	0.6

Table 6: Repeatability data of selpercatinib

S. No.	Area of selpercatinib
1	369650
2	365025
3	367566
4	365914
5	367584
6	363307
Mean	366508
S.D	2233.7
% RSD	0.6

Table 7: Intermediate precision data of selpercatinib

S. No.	Area of selpercatinib
1	375744
2	371751
3	374954
4	372564
5	373376
6	365756
Mean	372358
S.D	3556.3
% RSD	1.0

Table 8: Robustness data for Selpercatinib

S. No.	Condition	% RSD
1	Mobile phase (+)55A:45B	1.1
2	Temp. (+) 35°C	0.3
3	Flow rate (+) 1.1ml/min	0.3
4	Mobile phase (-)35A:65B	0.9
5	Temp. (-) 25°C	0.3
6	Flow rate (-) 0.9ml/min	0.3

Table 9: Specificity data of Selpercatinib

S.No.	Sample details	Retention time (min)
1	Placebo solution	Interference is not detected
2	Blank solution	Interference is not detected
3	Selpercatinib	2.647

Table 10: Assay data of Selpercatinib

S.No.	Sample area	Standard area	% recovery
1	369650	369172	99.93
2	365025	368855	98.68
3	367566	366301	99.36
4	365914	370186	98.92
5	367584	372102	99.37
6	363307	366276	98.21
Avg	366508	368815	99.08
Std dev	2233.7	2261.8	0.604
% RSD	0.6	0.6	0.61

Table 11: Degradation data of Selpercatinib

S. No.	Degradation condition	% Drug degraded	% Drug Undegraded	Retention time	Peak Area	Theoretical plates (>2000)
1	Acid	5.87	94.13	2.540	348221	3637
2	Alkali	4.99	95.01	2.630	351458	4027
3	Oxidation	4.06	95.94	2.623	354902	3668
4	Thermal	2.95	97.05	2.618	359023	3873
5	Photostability	1.77	98.23	2.622	363384	3575
6	Neutral	1.11	98.89	2.628	365814	4002

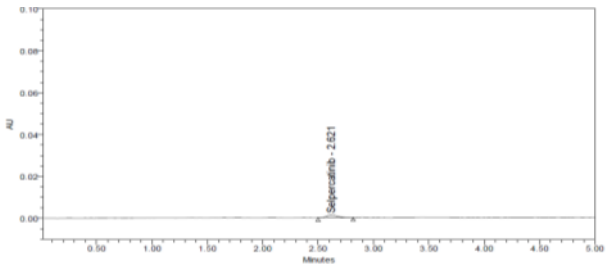

Figure 5: LOQ of Selpercatinib

satisfactory for the analysis of selpercatinib. The parameters for the selpercatinib have revealed that the theoretical plates were > 2000, and the tailing factor was < 2 (Table 2), (Figure 2).

Linearity

The developed method demonstrated linearity in the concentration range of 5- 30μg/ml. Linearity equations obtained for selpercatinib was found to be y = 18428x + 2196.2 with a correlation coefficient of 0.999. The high value of the correlation coefficient indicates good linearity. Results were tabulated in Table 3, graphically depicted in Figure 3.

Accuracy

Three levels (50%, 100%and 150%) of accuracy samples were made using the standard addition method. The % recovery was obtained in the range of 98.61%-100.87%. High recovery results obtained from the method shows that the suggested method can be utilised for QC analysis of capsule dosage forms. Results were represented in Table 4.

Precision

Chromatogram data for system precision (Table 5) revealed that % RSD was found to be 0.6, which was within the limit specified (%RSD NMT 2.0%). Chromatogram data for repeatability (Table 6) revealed that % RSD was found to be 0.6, which was within the limit specified (%RSD NMT 2.0%). Chromatogram data for intermediate precision (Table 7) revealed that % RSD was found to be one which was within the limit specified (%RSD NMT 2.0%). Hence, it proved the method was found to be precise.

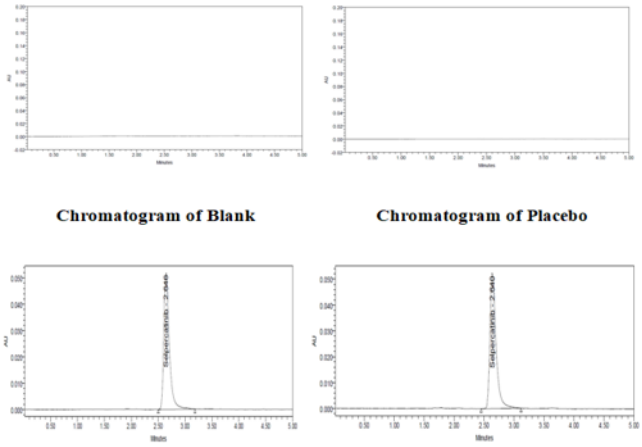

Figure 6: Chromatograms of blank, placebo, standard and sample

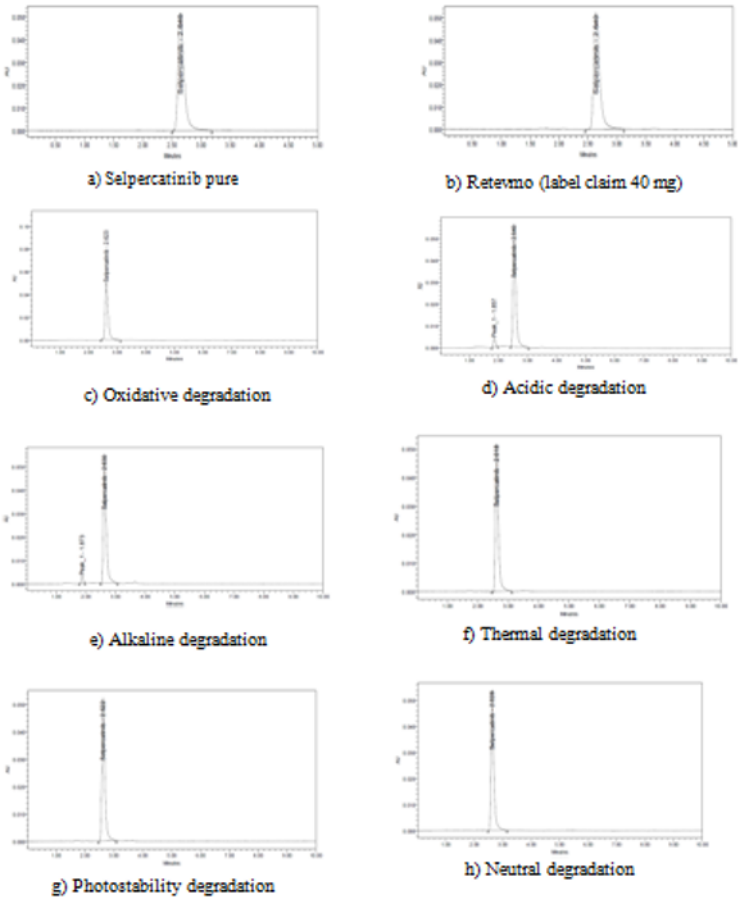

Figure 7: Typical chromatograms of forced degradation studies

Table 12: Summary Table

Parameters		Values	Limit
Linearity (μg/ml)		5-30μg/ml	
Regression coefficient		0.999	
Slope(m)		18428	R< 1
Intercept(c)		2196.2	
Regression equation (Y=mx+c)		y = 18428x + 2196.2	
Specificity		Specific	No intrusion of any peak
Accuracy (%recovery)		99.74%	98-102%
System precision (%RSD)		0.6	NMT 2.0%
Intermediate precision (%RSD)		1	
Repeatability (%RSD)		0.6	
Assay (% mean assay)		99.08%	90-110%
LOD		0.02 μg/ml	NMT 3
LOQ		0.05 μg/ml	NMT 10
Robustness	Mobile phase (+) 45B: 55A	1.1	%RSD NMT 2.0
	Flow rate (+) 1.1ml/min	0.3	
	Temperature (+) 35°C	0.3	
	Mobile phase (-) 65B: 35A	0.9	
	Flow rate (-) 0.9ml/min	0.3	
	Temperature (-) 25°C	0.3	

Sensitivity

LOD of selpercatinib was detected to be 0.02 μg/ml. LOQ of selpercatinib was detected to be 0.05μg/ml. Chromatograms of Limit of Detection and Limit of Quantification were demonstrated in Figure 4 and Figure 5, respectively.

Robustness

The robustness was assessed by introducing little, intentional variations in the chromatographic conditions, which comprise the flow rate of mobile ($\pm$ 0.1 ml/min), % of acetonitrile in the mobile phase (35 & 45%) and temperature($\pm$5).

Robustness was carried out using 10 μg/ml of selpercatinib and the % RSD was found to be 0.3-1.1. Data was represented in Table 8.

Specificity

Specificity was performed by introducing a blank solution, placebo solution, selpercatinib standard and sample solutions. Data represented in Table 9 (Figure 6) indicates that was no interference in the placebo and blank sample at the retention time of the standard selpercatinib sample. Therefore, the method was specific.

Assay

Retevmo (label claim 40mg) was used for the assay. The percentage purity of selpercatinib was found to be 99.08-99.93 in pharmaceutical formulations. Data was represented in Table 10.

Forced degradation studies

Selpercatinib was eluted at 2.647 min. Selpercatinib has undergone acid degradation (5.87%), alkali degradation (4.99%), oxidation (4.06%), thermal degradation (2.95%), photolytic degradation (1.77%) and neutral degradation (1.11%), which is less than 10%, indicating that the selpercatinib is more resistant towards all forced degradation conditions applied.

During acid degradation and alkali degradation, an extra peak was observed at 1.1857 min and 1.1873, along with the drug peak at 2.640 min (Table 11; Figure 7). The system suitability parameters were within limits.

CONCLUSION

Validated RP-HPLC method has been established for the quantification of selpercatinib in pure and its capsule dosage forms. The developed method was found to be precise, specific and accurate. Hence, the method can be utilised in the quality control analysis of selpercatinib.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

Adison, F and Sanjay Pai, P. N. 2019. A validated stability-indicating RP-HPLC method for estimation of tolfenamic acid in the presence of its pharmacopoeial impurities. *International Journal of Applied Pharmaceutics*, 11(5):264–270.

AnjaneyuluReddy, B., Radhakrishnanand, P., IrshadAlam, M., RaviKiran, P. 2019. A Validated Stability Indicating RP-HPLC Method Development for Anticancer Drug Enzalutamide in Bulk and Pharmaceuticals. *International Journal of Pharmaceutical Sciences and Drug Research*, 11(3):85–90.

Anthony, M. 2020. Selpercatinib: First Approval. *Drugs*, 80(11):1119–1124.

Bhanu, P., Sundararajan, R., Prathyusha, P., Mukthinuthalapati, M. A. 2020. A new stability indicating RP-UFLC method for determination of Luliconazole in bulk and pharmaceutical formulation. *Research Journal of Pharmacy and Technology*, 13(6):2859–2863.

Chinmaya, K. S., Sudhakar, M., Nalini, K. S., Ram, M. R. S. 2018. Validation of analytical methods: a review. *Journal of Innovation in Pharmaceutical Sciences*, 2(1):8–15.

Diksha, R., Vaishnav, R., Anju, G. 2013. Validation of Analytical Methods for Pharmaceutical Analysis. *International Journal of Pharmaceutical Education*, 3(1):31–40.

Goto, K., Oxnard, G. R., Tan, D. S. W., Loong, H. H. F., Bauer, T. M., Gainor, J. F., McCoach, C. E., Gautschi, O., Besse, B., Cho, B. C., Peled, N., Weiss, J., Kim, Y., Ohe, Y., Horiike, A., Park, K., Huang, X., Olek, E., Subbiah, V., Drilon, A. E. 2020. Selpercatinib (LOXO-292) in patients with RET-fusion+ non-small cell lung cancer. *Journal of Clinical Oncology*, 38(15):3584–3584.

Latha, S. T., AnandaThangadurai, S., Jambulingam, M., Sereya, K., Kamalakannan, D., Anilkumar, M. 2017. Development and validation of RP-HPLC method for the estimation of Erlotinib in pharmaceutical formulation. *Arabian Journal of Chemistry*, 10:S1138–S1144.

Manikandan, K., Lakshmi, K. S. 2012. Method development and validation of Risperidone by RP-HPLC. *International Journal of Research in Pharmaceutical and Nano Sciences*, 1(1):87–95.

Mathrusri, A. M., Yasaswini, S. R., Sai, S. A. 2019. New Stability Indicating RP-UFLC Method for the Determination of Bumetanide - A Potent Diuretic. *Acta Scientific Pharmaceutical Sciences*, 3(8):2581–5423.

Raja, A. M., Anusha, S., David, B., Rao, K. N. V., Selvakumar, D. 2015. Analytical method development and validation of anticancer drugs (imatinib and cabacitabine) by RP-HPLC method. *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 3(2):51–65.

Şenturk, R., Wang, Y., Schinkel, A. H., Beijnenjos, H., Rolf, W. S. 2020. Quantitative bioanalytical assay for the selective RET inhibitors selpercatinib and pralsetinib in mouse plasma and tissue homogenates using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, 1147(122131):1–8.

Wang, W., Shi, L., Jin, L., Wang, K. 2020. Determination of selpercatinib, a RET kinase inhibitor in rat plasma and its application to a pharmacokinetic study. *Biomedical Chromatography*. e5052.