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ORIGINAL ARTICLE

Development and Validation of UV-Spectrophotometric Method for Estimation of Selpercatinib

Navya Singamsetty and Raja Sundararajan*
Department of Pharmaceutical Analysis, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh - 530045, India.
*Corresponding author's Email: sraja61@gmail.com

ABSTRACT
The aim of the study was to establish an easy, precise and accurate UV spectrophotometric technique for quantitative estimation of selpercatinib in pure and capsule dosage forms.0.1 N HCl was used as a solvent. Selpercatinib maximum absorbance was observed (λmax) at 222 nm and linearity was in the range of 2-12 µg/ml. Linearity equations for selpercatinib was y=0.0659x +0.0023with correlation coefficient 0.9997. Percentage recovery was found between 98.33-99.75%. The percent relative standard deviation for intraday precision and interday precision were found to be less than two. The LOD and LOQ for selpercatinib were obtained as 0.05 and 0.15µg/ml. The spectrometric technique was validated as per ICH guidelines and was detected to be applicable for routine quantitative analysis of selpercatinib in pure and capsule dosage forms.
Keywords: *Selpercatinib, ICH guidelines, UV spectrophotometric method, Validation.*

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INTRODUCTION

Selpercatinibis chemically 6-(2-hydroxy-2-methyl propoxy)-4-[6-[6-[(6-methoxypyridin-3-yl)methyl]-3,6-diazabicyclo[3.1.1]heptan-3-yl]pyridin-3-yl]pyrazolo[1,5-a]pyridine-3-carbonitrile. The molecular formula is $C_{29}H_{31}N_7O_3$with molecular weight 525.6 g/mol. It is a kinase inhibitor with increased selectivity for rearranged during transfection (RET) tyrosine kinase receptors (RTKs) above the additional RTK classes [1]. It is used for the treatment of non-small cell lung cancer (NSCLC) and metastatic RET fusion-positive thyroid cancer[2].RET is a transmembrane receptor tyrosine kinase consisting of intracellular domains and extracellular transmembrane.RET activity is necessary for development of the nervous system and kidney. Selpercatinib is primarily metabolized in the liver by CYP3A4. 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/MS methods[3, 4] and RP-HPLC method [5]were reported. Very limited methods have been reported for selpercatinib determination. The aim of the study was to establish an easy, specific and accurate UV spectrophotometric technique for the quantification of selpercatinib in pure and its capsule dosage form.

MATERIAL AND METHODS

Instruments

T60U UV-VIS spectrophotometer was utilised for the study. The analysis was done by using UVWin 5 software. An electronic balance was used for measuring purpose. Pipettes and volumetric flasks of borosilicate glass were utilised in the study. All statistical calculations were doneutilising Microsoft Excel analytical tool2007.

Chemicals and reagents

Selpercatinib sample was attained as a gift sample from spectrum pharma research solutions (Hyderabad, India). All the chemicals utilised were of analytical grade.

Preparation of drug stock solution

Ten mg of selpercatinib was precisely weighed and transferred to volumetric flask (10ml). Then volume was made up with methanol to attain a drug stock solution of 1000µg/ml concentration.

Preparation of working standard solution

1ml drug stock solution was taken into volumetric flask (10 ml). Then volume was adjusted with 0.1N HCl to attain 100µg/ml concentration.

Preparation of 0.1NHCl

HCl (8.5ml) was dissolved in 100ml distilled water and the total volume was made up to 1litre in a standard1000ml volumetric flask.

Preparation of calibration curve

Six volumetric flasks (10 ml) were taken. From working standard solution 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml samples were taken into volumetric flasks (10 ml)and diluted with0.1N HCl to produce 2-12 µg/ml. From 200-400 nm UV range the solutions were scanned using UV-Visible spectrophotometer.

Assay

Selpercatinib (Brand name: Retevmo 40 mg) was used for assay. 20 capsules were weighed, an amount equivalent to ten mg was transeferred into a volumetric flask(10 ml)& dissolved in methanol. For 10 minutes, flask was sonicated. Then solution was filtered and made up with methanol. Aliquots of sample solutions were taken into 10 ml volumetric flask. The volume was adjusted with 0.1N HCl. Absorbance was measured at 222 nm.

Method validation

Linearity

The linearity is defined as its capability to get test results that are proportional to analyte concentration in samples within an appropriate range. Appropriate volume of aliquots from selpercatinib working standard solution were transferred to volumetric flask (10ml). The volume was adjusted with 0.1N HCl to give solutions (2- 12µg/ml). The absorbance of each solution was measured and a calibration curve was established by plotting absorbance versus concentration [6].

Accuracy

Accuracy is the closeness of agreement of measured value to the accurate value. Accuracy was performed at 50%, 100% and 150% by adding an acknowledged amount of standard stock solution of selpercatinib to the sample stock solution. The recovery was verified by estimation of drug in triplicate preparations at each specified concentration level and% RSD was calculated [7].

Precision

Precision is closeness of agreement between individual test results when a technique is subjected to several samplings of a uniform sample. Precision was studied by intraday and interday variation. In intra-day study, on the same day4, 8, 12µg/ml concentrations of drug were analysed for three times. In inter-day study the drug concentrations were analysed on 3consecutive days which shows the laboratory variation in dissimilar days. Percentage RSD was determined [8].

Sensitivity

Sensitivity was determined with respect to Limit of quantification and Limit of detection. LOD is the smallest concentration of analyte in a sample, which can be detected but not essentially quantified whereas LOQ is the smallest concentration of an analyte, which might be quantified with appropriate precision and accuracy [9].

LOD and LOQ for the assay were estimated utilising the subsequent formulas [10].

LOD = 3.3× standard deviation of response / slope of the calibration curve

LOQ = 10× standard deviation of response / slope of the calibration curve

Robustness

Robustness is an estimate of its capability to remain unchanged by little but intentional changes in parameters of analytical technique and gives a suggestion of its consistency throughout usage. It was performed by changing wavelength (±2 nm) [11].

RESULTS AND DISCUSSION

The developed technique was validated according to ICH guidelines. The developed technique demonstrated linearity in the range of 2- 12µg/ml. Linearity equations obtained for selpercatinib were found to be y = 0.0659x +0.0023with correlation coefficient 0.9997. Linearity data was demonstrated in Table 1. Overlain spectra and calibration graph of selpercatinib were shown in figure 2 and 3.Table 2 shows the results of % recovery for accuracy studies. Mean percentage recovery was found between

98.33% - 99.75%.The limits of mean % recovery are 98%-102%. The observed data were within the range which indicates good recovery values and accuracy of the developed analytical method. Table 3 and 4 shows % RSD for precision studies. Percentage relative standard deviation for intraday precision and interday precision were found between 0.25-0.71 and 0.29-0.85. The RSDs were found to be less than 2% and that indicates that the method was precise. Robustness data was represented in Table 5.% RSD were found to be 0.76 and 1.11. The data shows that percentage RSD values were well within the limit. Percentage purity was found to be 98.79% in assay data (Table 6). The results of all validation parameters were found within the standard limits and summarized in Table 7.

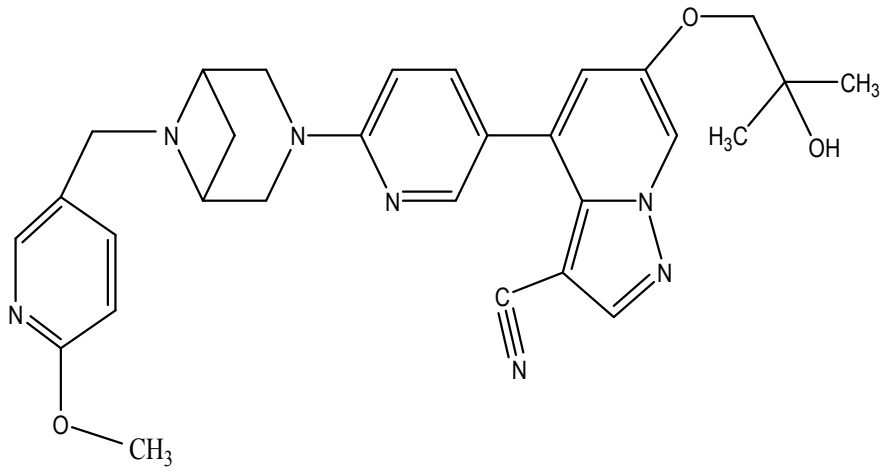

Figure 1: Structure of Selpercatinib

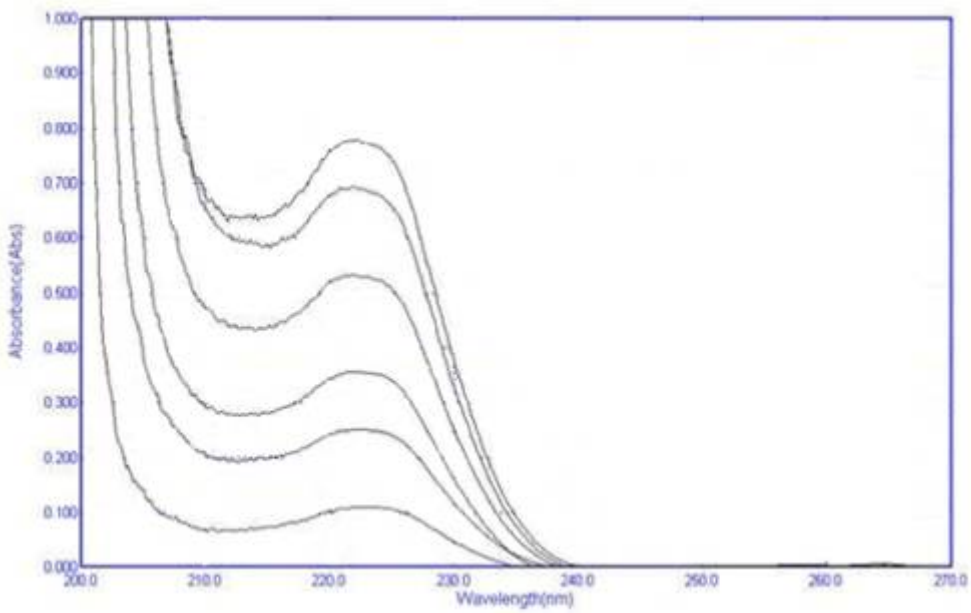

Figure 2: Overlain spectra of Selpercatinib

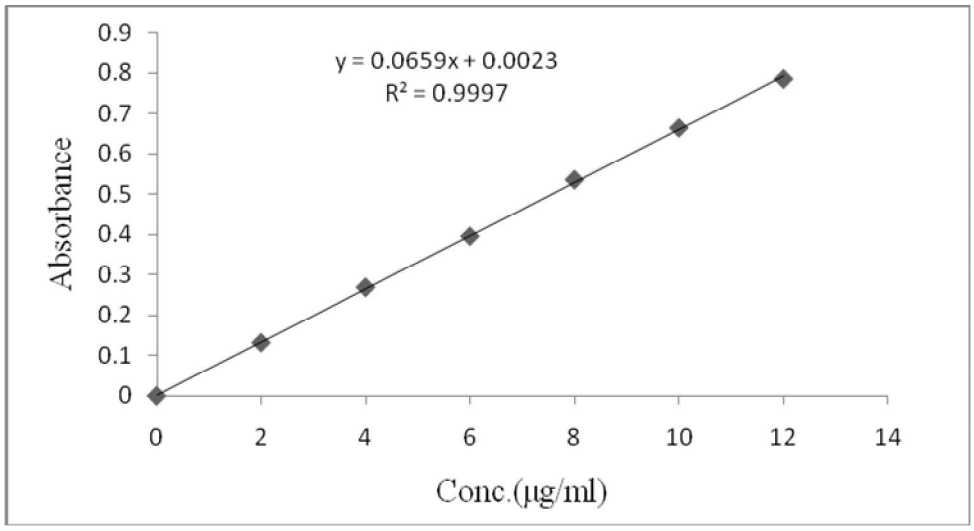

Figure 3: Calibration curve of Selpercatinib

Table 1: Linearity data of selpercatinib

Conc. (µg/ml)	Absorbance
2	0.132
4	0.269
6	0.396
8	0.536
10	0.665
12	0.786

Table 2: Accuracy data of selpercatinib

% of recovery level	Drug in capsule (µg/ml)	Pure drug added (µg/ml)	Drug recovered (µg/ml)	*Recovery (%) ± SD	RSD (%)
50%	8	4	11.8	98.33±0.83	0.84
100%	8	8	15.96	99.75±0.49	0.49
150%	8	12	19.83	99.15±0.39	0.39

*Mean of three determinations at each level

Table 3: Results of Intra-day precision

Concentration (µg/ml)	Concentration found (µg/ml)	*Assay±SD, RSD(%)
4	3.94	98.58±0.38, 0.38
8	7.96	99.37±0.25, 0.25
12	11.85	98.5±0.708, 0.71

*Mean of three determinations at each level

Table 4: Results of inter-day precision

Concentration (µg/ml)	Concentration found (µg/ml)	*Assay±SD, RSD(%)
4	3.93	98.33±0.28, 0.29
8	7.85	98.54±0.62, 0.63
12	11.73	98.19±0.85, 0.85

*Mean of three determinations at each level

Table 5: Robustness data of Selpercatinib

Wave length (nm)	Concentration (µg/ml)	Absorbance	Wave length (nm)	Concentration (µg/ml)	Absorbance
220	8	0.532	224	8	0.542
	8	0.523		8	0.541
	8	0.525		8	0.531
	Average	0.526		Average	0.538
	SD (±)	0.004		SD (±)	0.006
	(%) RSD	0.76		(%) RSD	1.11

Table 6: Assay data of Selpercatinib

Brand name	Available form	Label claim	Concentration found	Assay
RETEVMO	Capsule	40 mg	39.51	98.79

Table 7: Summary of validation parameters

Parameters	Obtained values
Maximum absorbance (λ max)	222
Linearity (µg/ml)	2-12
Molar absorptivity ($Mole^{-1}cm^{-1}$)	34777.2
Sandell's sensitivity ($\mu g/cm^2/0.001$)	0.015
Intercept(c)	0.0023
Slope(m)	0.0659x
LOD (µg/ml)	0.05
LOQ (µg/ml)	0.15
Intra-day precision (%RSD)	0.25-0.71
Inter-day precision (%RSD)	0.29-0.85
Recovery (%)	98.33-99.75

CONCLUSION

The proposed technique was validated by determining its precision, accuracy, robustness, sensitivity and specificity which proves the suitability of the technique for the routine analysis of selpercatinib.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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