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BIO ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TEPOTINIB IN RAT PLASMA WITH LC-MS/MS

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ABSTRACT

A highly responsive and simple lc-ms/ms assay was developed and witnessed for the gradation of tepotinib in rat plasma. The chromatographic conditions involve isocratic mode using waters symmetry c18 (150x4.6 mm, 3.5 µ) column. A mobile phase of 0.1% opa (ortho phosphoric acid) and acetonitrile in 50:50 is used and the detection was carried out in a +ve mode of electro spray ionization by using ms and entrectinib was used as internal standard. The lc-ms/ms method and validated in accordance with usfda guidelines.

Key words: tepotinib, lc-ms/ms, usfda guidelines, validation, rat plasma.

I. Introduction

Tepotinib, sold under the brand name tepmetko, is a medication for the treatment of adults [1,2] with metastatic non-small cell lung cancer (nsclc) [3,4,5] whose tumors [6,7] have a mutation that leads to mesenchymal-epithelial transition (met) [8,9] exon 14 skipping. It is a c-met inhibitor [10,11], a type of tyrosine kinase inhibitor [12,13]. Tepotinib is indicated for the treatment of adults with metastatic non-small cell lung cancer (nsclc) whose tumors have a mutation that leads to mesenchymal-epithelial transition (met) exon 14 skipping. The most common side effects seen in clinical trials were edema [14], fatigue [15], nausea [16], diarrhea [17,18], muscle aches, and shortness of breath [19]. Like capmatinib, tepotinib can also cause interstitial lung disease [20] and liver damage, and is toxic to a developing fetus.

II. Materials and methods

Chemicals and reagents

Acetonitrile and ortho phosphoric acid, water (hplc grade) were purchased from merck (india) ltd, worli, mumbai, india. Api of tepotinib as reference standard was procured from spectrum pharma research solutions pvt ltd, hyderabad.

Equipment

An hplc system (waters alliance e2695 model) connected with mass spectrometer qtrap 5500 triple quadrupole instrument was used. Data processing was performed with sciex software.

Chromatographic conditions

Chromatographic separation was carried out in isocratic mode at room temperature using symmetry c_{18} column (150x4.6 mm, 3.5 µ). A mixture of acetonitrile and 0.1% opa in 50: 50 v/v at a flow rate of 1.0 ml/min was used as mobile phase. The injection volume was 10 µl. The run time was 5 min.

Preparation of standard and quality control samples

Preparation of tepotinib stock

5 mg of tepotinib standard was accurately weighed out and dissolved in 100 ml of diluent (mobile phase). The concentration of the solution is 50 µg/ml. Take 0.8 ml of the above solution and diluted to 10 ml with diluent. This is the parent stock solution of tepotinib and the concentration of the parent stock solution is 4µg/ml. Take 0.4 ml of the above solution and diluted to 10 ml with diluent. This is the stock solution with concentration 160 ng/ml.

Preparation of entrectinib stock solution (internal standard)

5 mg of entrectinib standard was accurately weighed out and dissolved in 100 ml of diluent (mobile phase). The concentration of the solution is 50 µg/ml. Take 1 ml of the above solution and diluted to 10 ml with diluent. This is

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the parent stock solution of entrectinib and the concentration of the parent stock solution is 5 µg/ml. Take 0.4 ml of the above solution and diluted to 10 ml with diluent. This is the stock solution with concentration 200 ng/ml.

Preparation of standard solution

Standard solution was prepared by taking 0.5 ml of stock solution, 0.5 ml of is stock solution, 0.2 ml of plasma, 0.3 ml of acetonitrile and 0.5 ml of diluent in a centrifuged tube and vortexes for 15 min to mix the constituents. After centrifuge for 15 min, at 5000 rpm supernatant managed solution was separated and filtered through 0.45 µ nylon syringe filter into a vial and injected into lc-ms/ms system.

Preparation of sample solution

Preparation of sample stock

One tablet (contains 225 mg of tepotinib) was weighed, note the average weight of the tablet. The tablet was taken into a mortar and crushed into fine powder. 11.9 mg of tablet powder was weighed accurately and dissolved in 100 ml of diluent. From this take 0.8 ml and diluted to 10 ml with diluent. From this take 0.4 ml and diluted to 10 ml with diluent. This is the sample stock tepotinib.

Preparation of sample solution

For sample preparation take 0.2 ml of plasma, 0.5 ml of sample stock, 0.3 ml of acetonitrile and 0.5 ml of is, 0.5 ml of diluent were taken into a centrifuge tube and vortexed for 15 min to precipitate all the proteins. Centrifuge it for 15 min at 5000 rpm and collect the supernatant solution into a vial and inject it into lc-ms/ms system.

Method validation

Selectivity

Selectivity was performed by analyzing the rat plasma samples from six different rats to test for interference at the retention time of analytes.

Matrix effect

Matrix effect for tepotinib was evaluated by comparing the peak area ratio in the post extracted plasma sample from six different drug free blank plasma samples and neat reconstitution samples. Experiments were performed at mqc levels in triplicate with six different plasma lots with the acceptable precision of ≤ 15%.

Precision and accuracy

It was determined by replicate analysis of quality control samples (n=6) at a lower limit of quantification (lloq), low quality control (lqc), medium quality control (mqc), high quality control (hqc) levels. The % cv should be less than 15% and accuracy should be within 15% except lloq where it should be within 20%.

Recovery

The extraction efficiencies of tepotinib were determined by analysis of six replicates at each quality control concentration. The percentage recovery was evaluated by comparing the peak areas of extracted standards to the peak areas of un extracted standards.

Carry over

The analyte retained by the chromatographic system during the injection of a sample that appears in subsequent blank or unknown samples.

Dilution integrity

Dilution integrity should be demonstrated by spiking the matrix with an analyte concentration above the uloqc and diluting this sample with blank matrix.

Stability

Stock solution stability was performed by comparing the area response of analyte in the stability sample with the area response of sample prepared from fresh stock solution. Stability studies in plasma were performed at the lqc and hqc concentration levels using six replicates at each level. Analyte was considered stable if the change is less than 15% as per us fda guidelines. The stability of spiked rat plasma samples stored at room temperature was evaluated for 24 hrs. The stability of spiked rat plasma stored at 2-8°c in auto sampler was evaluated for 24 hrs. The auto sampler stability was evaluated by comparing the extract plasma samples that were injected immediately, with the samples that were re-injected after storing in the auto sampler at 2-8°c for 24 hrs. The reinjection reproducibility was evaluated by comparing the extracted plasma samples that were injected immediately, with the samples that were re injected after storing in the auto sampler at 2-8°c for 24 hrs. The freeze thaw stability was conducted by comparing the stability samples that had been frozen at -30°c and thawed three times, with freshly spiked quality control samples. Six aliquots each of lqc and hqc concentration levels were used for the freeze thaw stability evaluation. For long term stability evaluation the concentrations obtained after 24 hrs were compared with initial concentration.

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III. Results and discussion

Electro spray ionization having maximum response over atmospheric pressure chemical ionization mode selected in this method. The optimization of instrument to give sensitivity and signal stability during in fusen of the analyte in the continuous flow of the mobile phase to electro spray ion source operated at both polarities at flow rate of 10 µl/min tepotinib give more response in positive ion mode when compared with negative ion mode. Standard chromatogram was shown in figure 1.

Specificity

Interfering peaks were not observed at tepotinib and entrectinib (is) retention times in the chromatogram of blank rat plasma. Thus proved specificity of the method to analyze tepotinib. Figure 2 shows the blank chromatogram.

Matrix effect

In matrix effect six lots of rat plasma at lqc and hqc of tepotinib were 99.5 and 99.8. % cv of the drug at lqc level were 0.83 and hqc level is 1.11 respectively. It indicates that the matrix effect on the ionization of the analyte is within the acceptable limit.

Linearity

The peak area ratios of calibration standards were proportional to the concentration. The concentration range of tepotinib was 4-80 ng/ml. The calibration curves were appeared linear and correlation coefficient was found to be 0.999 for tepotinib. Linearity result of tepotinib was shown in following table. Table 1 gives the results of linearity and figure 3 gives the calibration plot of tepotinib.

Precision and accuracy

The precision and accuracy were determined by pooling all individual assay results of different quality control samples. The accuracy results of tepotinib in quality control samples 99.2-100.1. The % cv of tepotinib was < 5% in all quality control samples. Basing on the given data, it was clear that the method is precise and accurate. Precision and accuracy results were shown in table 2.

Recovery

The recoveries for tepotinib (98.67%-100.53%) at lqc, mqc and hqc levels and %cv ranged from 0.65-1.43. The results demonstrated that the bioanalytical method had good extraction efficiency. This also showed that the recovery was not dependent on concentration.

Ruggedness

The percent recoveries and percent cv of tepotinib determined with two different analysts and on two different columns were within acceptable criteria in hqc, lqc, mqc and llqc samples. The percent recoveries ranged from 98.36-100.48% for tepotinib. The %cv values ranged from 0.32-0.79 for tepotinib. The results proved method is ruggedness.

Auto sampler carryover

Peak area response of tepotinib and entrectinib was not observed in the blank rat plasma samples after successive injections of llqc and ulqc at the retention times of tepotinib and entrectinib. Therefore this method does not exhibit auto sampler carryover.

Stability

In solution stability analysis, tepotinib solutions were prepared with diluents and put in storage at 2-8°c in a refrigerator. Fresh stock solutions were related to stock solutions prepared earlier 24hrs. The % change of tepotinib was 1.12%, shows that stock solutions were stable up to 24 hrs when stored in 2-8°c. Bench top and auto sampler stabilities were observed at lqc and hqc levels. At room temperature tepotinib was stable in plasma for 24 hrs, and 24 hrs in auto sampler at 20°c. From this, it was confirmed that at lqc and hqc levels repeated freezing and thawing of plasma samples spiked with tepotinib did not affect its stability. From long term stability it was clear that tepotinib was stable up to 24 hrs at a storage temperature of -30°c. The overall stability results of tepotinib were tabulated in the following table.

IV. Conclusion

For the first time higher sensitive hplc-esi-lcms/ms method was developed and validated for the determination of tepotinib in rat plasma. Here the described method is rugged, fast, reproducible bio analytical method. Simple and efficient method was developed. This method was validated according to usfda guidelines.

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Table 1: results of linearity

Linearity	Tepotinib	
	Conc. (ng/ml)	Area response ratio
1	4.00	0.067
2	10.00	0.164
3	20.00	0.312
4	30.00	0.464
5	40.00	0.648
6	50.00	0.789
7	60.00	0.947
8	80.00	1.270
Slope	0.0157	
Intercept	0.00377	
Cc	0.9996	

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Table 2: precision and accuracy of tepotinib

Qc name	Llqc	Lqc	Mqc	Hqc
Conc. (ng/ml)	4	20	40	60
Qc sample-1	4.252	20.105	40.158	60.124
Qc sample-2	4.241	20.147	40.174	60.114
Qc sample-3	4.213	20.132	40.196	60.234
Qc sample-4	4.262	20.168	40.135	60.165
Qc sample-5	4.234	20.142	40.128	60.117
Qc sample-6	4.215	20.169	40.113	60.106
Mean	4.236	20.144	40.151	60.143
Sd	0.01965	0.02400	0.03108	0.04901
%cv	0.464	0.119	0.077	0.081
Accuracy	105.58	100.41	100.07	99.94

Table 3: stability results of tepotinib

Stability experiment spiked plasma		Spiked plasma conc. (n=6, ng/ml)	Conc. Measured (n=6, ng/ml)	% cv
Bench top stability	Lqc	20	20.036	1.02
	Mqc	40	40.157	0.65
	Hqc	60	60.038	0.49
Auto sampler stability	Lqc	20	20.362	0.98
	Mqc	40	40.336	0.35
	Hqc	60	60.187	0.54
Long term (day 28) stability	Lqc	20	19.953	1.16
	Mqc	40	40.231	1.01
	Hqc	60	59.998	0.58
Wet extract stability	Lqc	20	20.346	0.63
	Mqc	40	40.089	0.34
	Hqc	60	60.321	0.89
Dry extract stability	Lqc	20	20.014	0.27
	Mqc	40	40.157	1.14
	Hqc	60	60.221	0.09
Freeze thaw stability	Lqc	20	19.936	0.57
	Mqc	40	40.178	0.46
	Hqc	60	60.027	0.54
Short term stability	Lqc	20	20.042	0.99
	Mqc	40	40.110	0.43
	Hqc	60	60.029	0.21

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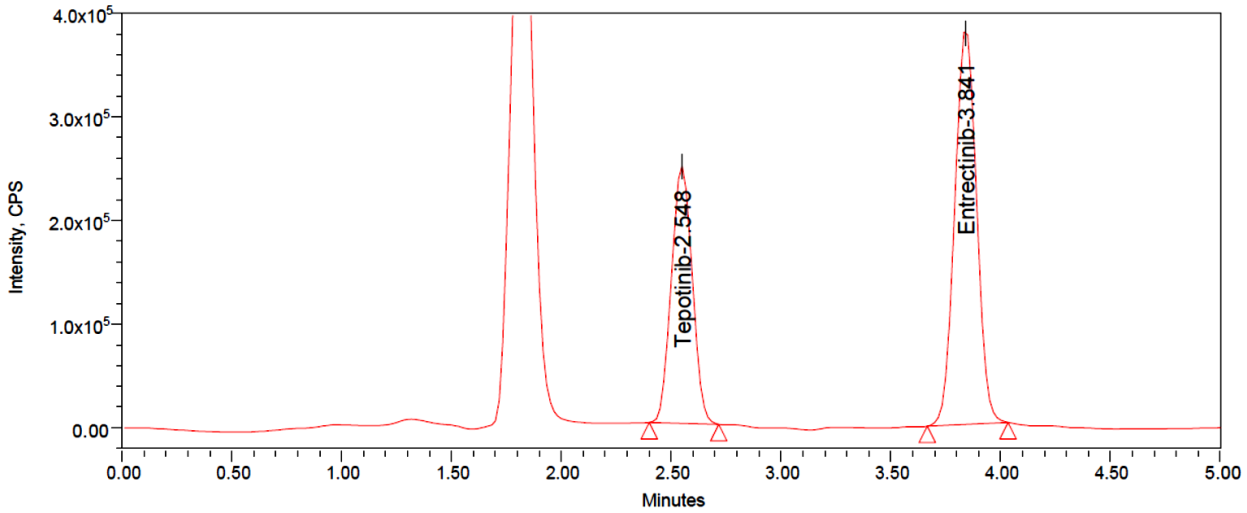

figure 1: chromatogram of standard

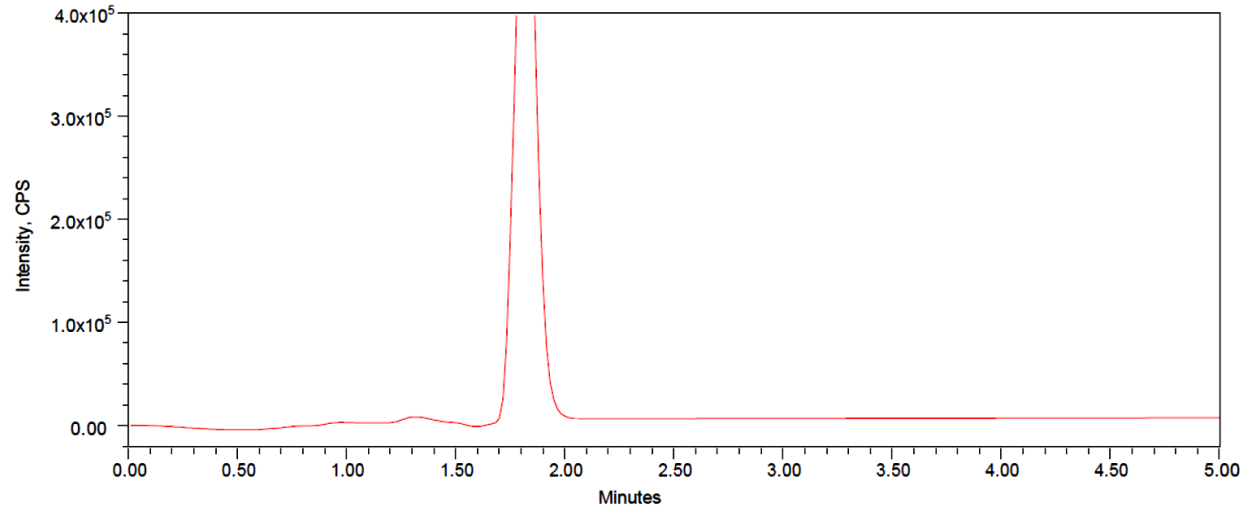

Figure 2: chromatogram of blank

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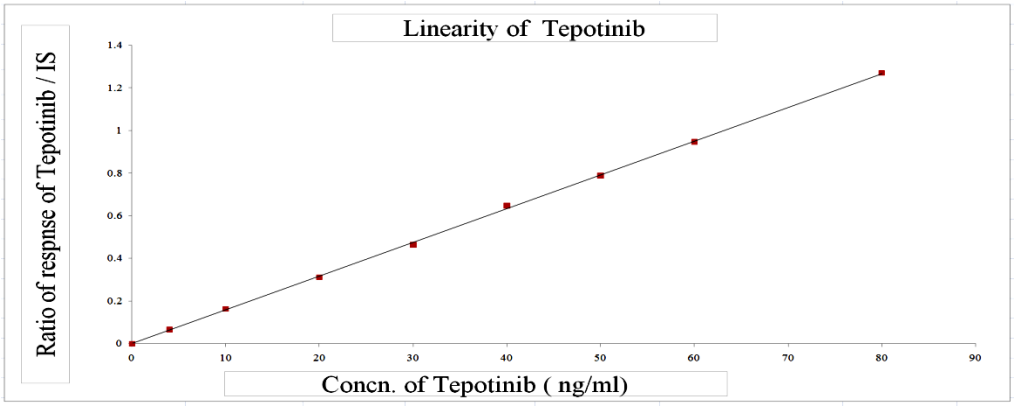

Figure 3: calibration plot of tepotinib