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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF MITAPIVAT IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Mitapivat in pharmaceutical dosage form. Chromatographic separation of Mitapivat was achieved on Waters Alliance-e2695, by using Zorbax SB C18 (250x4.6mm, 5µ) column and the mobile phase containing ACN and Water in the ratio of 80:20% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 278nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Mitapivat were NLT 2000 and should not more than 2 respectively. %Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Mitapivat.

Key words: *HPLC Method, Mitapivat, ICH guidelines, Validation, Degradation studies.*

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INTRODUCTION:

Haemoglobin levels in an individual fall below their baseline levels in anaemia; however, when baseline haemoglobin is unknown, sex- and race-specific reference ranges are frequently employed to make a diagnosis. The World Health Organisation (WHO) defines anaemia as having a haemoglobin level of less than 12g/dL in women and less than 13g/dL in males. In addition to age and race, there are new criteria for anaemia in both men and women who have experienced chemotherapeutic effects. Even "special populations" like smokers, sportsmen, senior citizens, or those who live at high elevations have recommended various ranges.[1-3]

Adults with pyruvate kinase (PK) deficiency can be treated for hemolytic anaemia with mitapivat, a pyruvate kinase activator. An essential enzyme necessary for red blood cell survival, erythrocyte pyruvate kinase, is made more active by mitapivat. Red blood cells cannot produce enough energy as a result of defects in the pyruvate kinase enzyme, which results in chronic hemolytic anaemia or permanent premature red blood cell lysis. .[4-6] Mitapivat chemically (fig-1)N-[4-[4-(cyclopropylmethyl)piperazine-1-carbonyl]phenyl]quinoline-8-sulfonamide.[7]

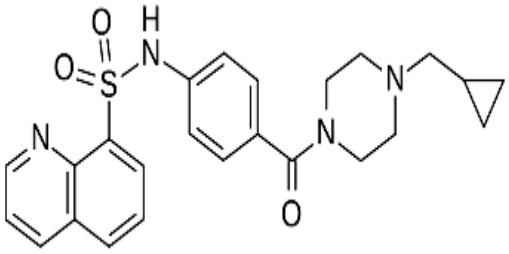

Fig 1: Structure of Mitapivat

Although a number of analytical techniques are described in the literature for the measurement of anemia medications, there are no HPLC techniques described for the estimation of Mitapivat. The goal of the current work is to provide a new, straightforward, accurate, exact, and affordable approach for simultaneously estimating Mitapivat and to validate the method using studies of forced deterioration in accordance with ICH recommendations. [8-10]

MATERILS AND METHODS:

Chemicals and reagents:

As a gift sample, Agios Pharmaceuticals, Inc. provided the reference and sample of Mitapivat (Pyrukynd -20mg). Water (HPLC grade) and acetonitrile (HPLC grade), ortho phosphoric acid (AR grade), sodium hydroxide (pure), and hydrogen peroxide (pure) were acquired from Merck Limited and Zodiac Life Sciences, respectively.

Instrumentation:

The WATERS alliance Quaternary pumps, a Photo Diode Array detector, and an integrated auto sampler are all features of the HPLC 2695 SYSTEM. UV-VIS spectrophotometer thermometer with matching quartz cells and a particular bandwidth of 2mm and 10mm, Electronics Balance-Denver pH meter-BVK enterprises, India Unichrome-UCA 701 ultrasonicator.

Optimized chromatographic conditions:

The chromatographic separation was performed on Zorbax SB C_{18} (250mmx4.6, 5µm) at an ambient column temperature. The samples was eluted using Acetonitrile: water (80:20) as the mobile phase at a flow rate of 1ml/min the mobile phase and samples were degassed by ultra sonication for 30 min and filtered through 0.45µm Nylon (N66) 47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1mL/min, and PDA detection was carried out at 278 nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Mitapivat was recorded under optimized chromatographic conditions. (Fig-2)

Standard solution preparation:

Precisely weigh and transfer 5 mg of the Mitapivat working standard into a 10 ml clean, dry volumetric flask. Add diluent, sonicate to thoroughly dissolve it, and then add enough of the same solvent to put the volume up to the target. (Stock solution), Added pipette in a 10 ml volumetric flask, add 1 ml of the aforementioned stock solutions and diluent to the desired concentration. (Mitapivat at 50ppm)

Preparation of sample solution:

Mitapivat sample was precisely weighed and transferred into a 10 mL clean, dry volumetric flask. Diluent was added, the sample was sonicated for up to 30 min to dissolve it, and then centrifuged for 30 min to thoroughly dissolve it and make volume up to the required level using the same solvent (Stock solution). Pipette 1 ml of the aforementioned stock solutions into a 10-ml volumetric flask and add diluent (containing 50 ppm of Mitapivat) to get the desired concentration.

Validation of developed method:

According to ICH recommendations for system appropriateness, specificity, recovery, accuracy, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ), the suggested technique

was validated. The following parameters were examined as part of the validation research.

System suitability:

The system suitability parameters were determined by preparing standard solutions of (50ppm) Mitapivat and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were calculated and results were tabulated table 1 and fig 2 and 3.

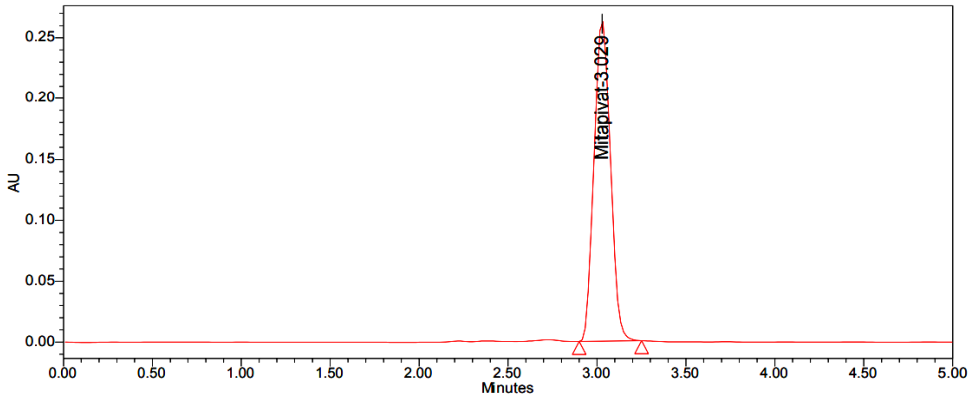

Fig 2: Optimized chromatogram of Mitapivat

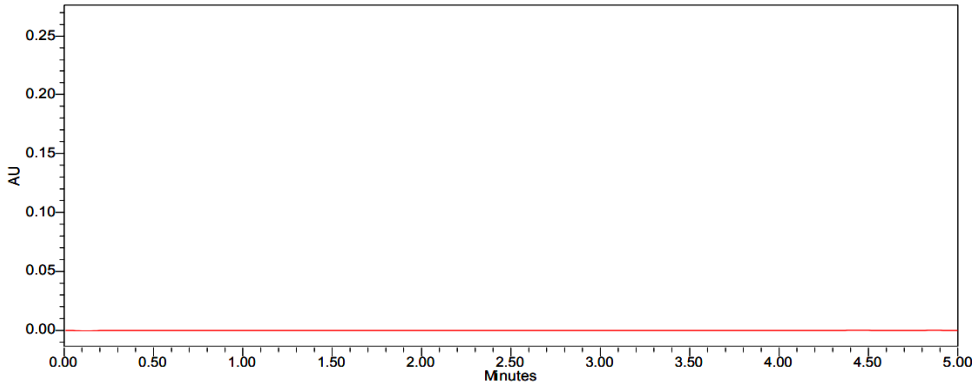

Fig 3: Chromatogram of blank

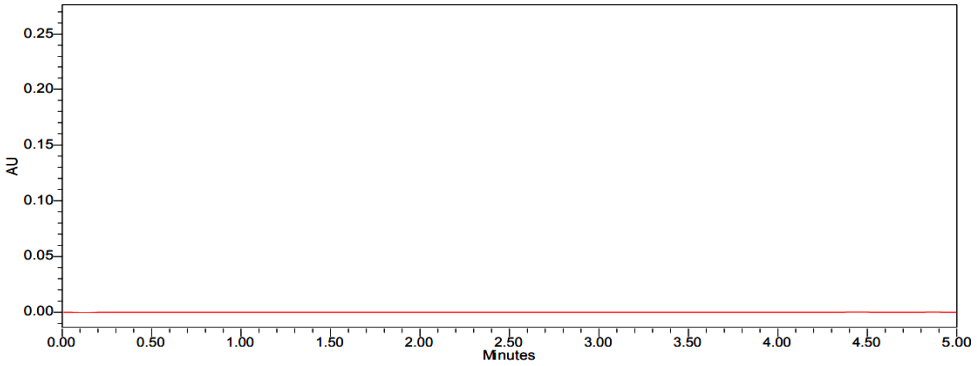

Fig 4: Chromatogram of placebo

Table 1: System suitability parameters for Mitapivat

S. no	Parameter	Mitapivat
1	RT	3.027
2	USP Plate count	11431
3	USP TF	0.98
4	% RSD	0.31

Specificity:
The specificity of the method was carried out to check whether there is any interference of any impurities with the retention time of analyte peaks. The specificity was performed by the injecting blank, Placebo and standard solutions of drugs and showed in fig 2-4.

Precision:
Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Mitapivat, have been analyzed by injecting them into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were taken and standard deviation, % relative standard deviation (RSD) was calculated and results were tabulate in table 2.

Table 2: precision data of Mitapivat

S. No	Method precision	Intermediate precision	
		Day 1	Day 2
1	2078974	2092889	2059684
2	2092417	2088794	2035211
3	2099810	2071815	2041279
4	2096608	2079611	2047220
5	2084295	2088397	2053652
6	2080406	2079058	2060195
Average	2088752	2083427	2049540
Standard Deviation	8747.093	7893.334	10122.105
% RSD	0.42	0.38	0.49

Accuracy:
The common addition technique is used to verify accuracy at three distinct levels: 50%, 100%, and 150%. At each level, a known quantity of the reference medication was added to the blank sample. Each sample received three injections. The computed and acceptable Mitapivat mean recovery rate was 100±2% and data were tabulated in table 3.

Table 3: Accuracy data of Mitapivat

%Con Level	Average Area	Amount Added(mg)	Average Amount Found(mg)	Average %Recovery	Mean% Recovery
50%	1035624	2.5	2.49	99.6	99.5
100%	2079487	5.0	4.99	99.8	
150%	3094873	7.5	7.43	99.1	

Linearity:
The linearity of the method was established by determining the absorbance of different concentrations of Mitapivat over a range of 12.5-75µg/ml respectively. Six replicates of each concentration were independently prepared and injected in to HPLC system. The linearity was determined by calculating a regression line from plot of peak area ratio of drug and is versus concentration of the drug. Regression analysis was computed for Mitapivat. The method was evaluated by determination of correlation coefficient and intercept values according to ICH guidelines and data was tabulated in table 4 and figure 5.

Table 4: Linearity data of Mitapivat.

S.No	Mitapivat	
	Coc of mitapivat	Area of peak
1	12.50	522856
2	25.00	1038964
3	37.50	1546354
4	50.00	2087836
5	62.50	2520412
6	75.00	3056381
Regre.equation	Y =40608.93x+16136.82	
Slope	40608.93	
Intercept	16136.82	
R^2	0.99978	

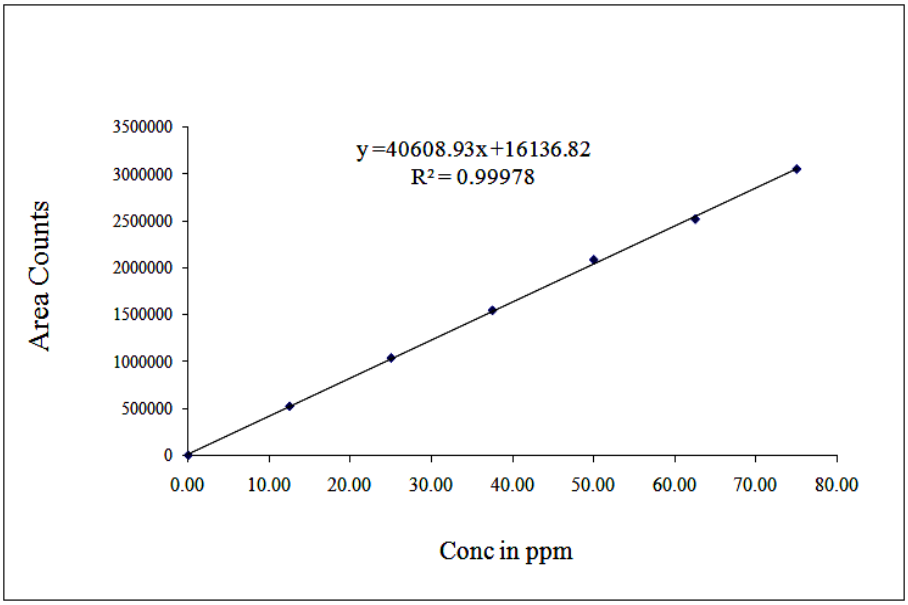

Fig 5: Calibration curve for Mitapivat

Limit of Detection and Limit of Quantification:

By using the calibration curve approach, the limits of detection (LOD) and quantification (LOQ) of Mitapivat were established. In triplicate, linearity-ranged solutions of Mitapivat were produced. Three analyses' average peak areas were displayed versus concentration. The following formulae were used to determine LOD and LOQ.

LOD= 3 x N/ B

LOQ= 10 x N/ B

Where N is residual variance due to regression; B is the slope.

LOD for Mitapivat was found to be 0.15µg/mL and LOQ for Mitapivat was found to be 0.5µg/ml.

Robustness:

To assess the analytical method's robustness, HPLC settings were slightly altered. These adjustments affected the flow rate, and change of organic phase in the mobile phase and results were tabulated in table 5.

Table 5: Robustness data of Mitapivat

Parameter	Mitapivat				
	Condition	RT (min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.9ml)	3.349	2261452	0.95	11536
	Actual(1ml)	3.029	2087836	0.93	11427
	More flow(1.1ml)	2.778	1945947	0.90	11345
Organic Phase change	Less Organic phase (72:28)	3.520	2357416	1.02	11581
	Actual (80:20)	3.027	2079606	0.99	11438
	More Organic phase (88:12)	2.838	1761411	0.94	11312

Degradation Study

The sample solutions were made direct exposure to UV light, alkaline, acidic, thermal, oxidative stress, and water testing. The research of forced deterioration didn't add any internal standards and results were tabulated in table 6.

Table 6: Forced Degradation data of Mitapivat

Degradation Condition	Mitapivat (1N)				
	Area	%Assay	%Degradation	Purity Angle	Purity Threshold
Control	2088749	100	0	0.784	2.965
Acid	1806478	86.4	13.6	0.732	2.955
Alkali	1829692	87.6	12.4	0.749	2.937
Peroxide	1776587	85.0	15.0	0.767	2.948
Reduction	1861287	89.1	10.9	0.751	2.926
Thermal	1876545	89.8	10.2	0.733	2.968
Photolytic	2011572	96.3	3.7	0.721	2.911
Hydrolysis	2074120	99.3	0.7	0.776	2.937

Assay:

Assay of the marketed tablet was carried out by injecting samples corresponding to equivalent weight into the HPLC system and recovery studies were carried out and results were tabulated in table 7.

Table 19: Assay of Mitapivat

Brand name	Name of drug	Peak Area	Average area	Std. wt. (mg)	Sample wt. (mg)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
Pyrukynd	Mitapivat	2089415	2081039	5	134	50	99.9	4.997	99.9
		2072663							

CONCLUSION:

The HPLC approach that has been devised for the estimation of certain pharmaceuticals is straightforward, quick, exact, reliable, and affordable. The mobile phase and solvents need less effort to prepare, are cost-effective, dependable, sensitive, and take less time. The sample recoveries revealed that formulation excipients did not interfere with the estimate and were in excellent accordance with the promises made on their separate labels. They may be utilized in laboratories for the regular examination of specific medications. Since the HPLC method's system validation parameters have produced satisfactory, accurate, and repeatable results (without the interference of excipients), it is inferred that the short and simple proposed methods will be the most helpful for analysis purposes. According to the results of the current study, the stability-indicating test

technique by RP-HPLC was straightforward, exact, and specific, and it didn't interact with the placebo or degradation products. As a result, these may be applied to regular analyses of Mitapivat.

Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication.

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