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ESTIMATION OF PAZOPANIB HYDROCHLORIDE IN TABLET DOSAGE FORMS BY RP-HPLC

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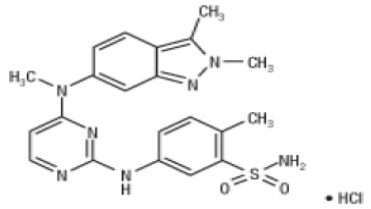
Abstract
A simple, precise, rapid and accurate RP- HPLC method was developed for the Estimation of Pazopanib HCl (PZP) in tablet dosage forms. An XTerra RP C_{18}, (250X4.6 with 5 microns particle size) and the mobile phase, consisting of 0.03M KH_2PO_4 in water adjusting the pH-3.2 with O-Phosphoric Acid: Acetonitrile in ratio of 70:30 v/v & water: Acetonitrile (50:50 v/v) was used as diluent in the gradient mode. The flow rate was 1.0 ml/min and the effluents were monitored at 267 nm. The retention time was 7.392 for Pazopanib HCl. The detector response was linear in the concentration of 20-240 µg/mL for PZP. The respective linear regression equation being Y (528142.1276 = 172694.049x + 428066.7611 for PZP. The Limit of Detection (LOD) is 0.10 & The Limit of Quantification (LOQ) is 0.30 for PZP respectively. The % assay of PZP was found out to be 99.49%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of PZP in bulk drug and in its pharmaceutical dosage forms.
***Keywords*:** Pazopanib HCl (PZP), RP-HPLC, Estimation, and Tablets

1. Introduction

Pazopanib HCl (PZP) chemically, 5-[[4-[(2, 3-dimethyl-2H-indazol-6- yl) methylamino]-2-pyrimidinyl] amino]- 2-methylbenzenesulfonamide monohydrochloride. **(Figure: 1)**.The empirical formula is $C_{21}H_{23}N_7O_2S$•HCl & the molecular weight is 473.99 gms/mol. It is a potent and selective multi-targeted receptor tyrosine kinase inhibitor of VEGFR-1, VEGFR-2, VEGFR-3,PDGFR-a/β[1], and c-kit that blocks tumor growth and inhibits angiogenesis[2]. It has been approved for renal cell carcinoma and soft tissue sarcoma[3-5] and also active in ovarian cancer[6]. Pazopanib also appears effective in the treatment of non-small cell lung carcinoma. Literature survey reveals a few chromatographic methods[7, 8] to determine PZP in tablet dosage form and also in biological fluids. HPLC methods are useful in the determination of drugs in pharmaceutical formulations, especially those containing more than one active component. From the literature, neither liquid chromatography methods nor assay methods have been reported for the estimation of PZP in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of PZP in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of PZP in bulk drug samples and also in pharmaceutical dosage forms.



Pazopanib HCl ((Figure: 1)

2. Experimental:

2.1 Materials/Chemicals and Reagents: Pazopanib HCl was obtained as a gift sample from M/s GlaxoSmithKline Pharmaceuticals Ltd. (GSK Rx India), Mumbai. Acetonitrile, Methanol and water used were of HPLC grade (Qualigens). Potassium Dihydrogen Orthophosphate and Ortho- Phosphoric Acid were obtained from SDFCL, Mumbai. Commercially available tablets (Votrient®- GSK Rx India) were procured from local market.

2.2 *Chromatography Instrument:* Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 10 µl, and 2693 pump. An XTerra, RP-C_{18} Column (250x4.6 mm i.d; particle size 5 µ) was used. The HPLC system was equipped with Empower 2 Software. The column

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was maintained at 40° C and eluted under isocratic conditions over 20.0 min at a flow rate of 1.0 ml/min.

2.3 HPLC Conditions: The contents of the Mobile Phase - consisting of 0.03M KH_2PO_4 in water adjusting the pH: 3.2 with O-Phosphoric Acid: Acetonitrile in ratio of 70:30 v/v & water: Acetonitrile in ratio of 50:50 v/v was used as diluent in the gradient mode. They were filtered before use, through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 20.0 min and the column temperature was ambient. Prior to the injection (20 µl) of the drug solution, the column was equilibrated for at least 30 min with the mobile phases flowing through the system. The eluents were monitored at 267 nm.

***2.4 Preparation of the Primary Standard/Stock Drug Solution*:** A standard stock solution of the drug was prepared by dissolving 100 mg of PZP in 50 ml volumetric flask containing 15 ml of diluent (Water: Acetonitrile in ratio of 50:50 v/v), sonicated for about 15 min and then made up to 50 ml with Methanol to get standard stock solution of 0.2 mg/mL of PZP.

***2.5 Preparation of the Working Standard Drug Solution*:** 5ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent (Water: Acetonitrile in ratio of 50:50 v/v) to get a concentration of 200 µg/mL of PZP respectively.

***2.6 Preparation of Sample solution*:** Twenty tablets (Votrient®- GSK Rx India) were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture containing concentration of each 0.2 mg/mL of PZP active ingredients, was mixed with 15 ml of Water: Acetonitrile in ratio of 50:50 v/v as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding Methanol up to 50 ml to obtain a stock solution. 5ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of 200 µg/mL of PZP.

2.7 Linearity: Aliquots of standard PZP stock solution was taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of PZP was in the range of 10-300 µg/mL. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 267 nm and the Calibration graph was obtained by plotting peak area versus concentration in µg/mL of PZP **(Figure: 2)**. The plot of peak areas of each sample against respective concentration of PZP was found to be linear in the range of 20-240 µg/mL with correlation coefficient of 0.998. Linear regression least square fit data obtained from the measurements are given in **Table I**. The respective linear regression equation being Y= 528142.1276x + 428066.7611 for PZP. The regression characteristics, such as slope, intercept, and % RSD were calculated for this method and given in **Table I.**

2.8 Accuracy: Accuracy was evaluated in triplicate by addition of three different amounts of PZP, to a previously analyzed sample and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 80, 100, and 120% of the amount originally present. %Recovery and RSD (%) were calculated for amount added. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in **Table: II**.

2.9 Precision: The precision of the method was ascertained, separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation.

The HPLC system was set up, describing chromatographic conditions, mentioned as above and following the system equilibration of the working standard solution containing 200 µg/mL of PZP, by injecting six times and recording the response peak areas. The precision was repeated with the formulated sample for the same concentrations by injecting the working sample solutions containing 200 µg/mL of PZP. The sample (Votrient®- GSK Rx India), was processed six times for the response of peak area. The % Relative Standard Deviation (RSD) and % range of error (at 0.05 and 0.01 confidence levels) were calculated and presented in **Tables: III & IV** respectively.

2.10 Limits of Detection and Quantitation: Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

2.11 Method Applicability: The present developed method was evaluated by applying to Pharmaceutical dosage forms for the estimation of PZP by our research group.

2.11.1 Assay: 20 µl of sample solution (Votrient®- GSK Rx India) was injected into the injector of liquid chromatography. The retention time was found to be 7.386 min for PZP. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table II**.

2.11.2 Recovery Studies: Accuracy was determined by recovery studies of PZP; known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of

recovery studies are shown in **Table II**. The study was done at three different concentration levels.

3. Results and Discussion

3.1 *HPLC Method Development and Optimization*[9]*:* In response to lack of simple, reliable and easy-to-use method for the determination of PZP concentrations in pharmaceutical matrices, an isocratic RP-HPLC method was developed for quantification of above mentioned, API. We examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of Methanol-Water, Methanol – Acetonitrile, Water – Acetonitrile & Ortho- Phosphoric Acid - Acetonitrile were tested. Ortho- Phosphoric Acid with Acetonitrile (70:30 v/v) was promisingly preferred, because it resulted in greater resolution of API after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were different column, temperature, variation in flow rate, detection wavelength, buffer pH variation in mobile phase and volume of injection. Buffer molarity was changed and optimum buffer strength was selected as 0.03M Potassium Dihydrogen Orthophosphate in water adjusted to pH 3.2, on the basis of theoretical plate number. At 267 nm, UV responses of all three active pharmaceutical analytes were good and free form interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram (Std & Working Sample) of PZP has been shown in **Figure: 5 & 6.**

The system suitability tests were carried out on freshly prepared standard stock solutions of PZP. Parameters that were studied to evaluate the suitability of the system were discussed and presented in **Table V**.

3.2 Method Validation Tests: Recommended method validation characteristics including Method precision (RSD, %), Method accuracy (Recovery % and RSD, %), Linear range (Correlation Coefficient), and LOD & LOQ, were investigated systematically.

3.3 Linearity: The plot of peak areas of each sample against respective concentrations were found to be linear, in the range of 20-240 µg/ml for PZP with Correlation Coefficient of 0.998 (**Table: 1)**. Linear regression least square fit data obtained from the measurements are given in **Table: 1.** The respective linear regression equation being Y= 172694.049x + 428066.7611 for PZP. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table I.** The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table: 1.**These results show that there was an excellent correlation between peak areas and analyte concentration.

3.4 Accuracy: Recovery of the individual substances at 80%, 100%, and 120% of specified concentrations were between 92.61% -109.57%, which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (**Table: II**)

3.5 Precision: The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (**Table: III & IV**).

3.6 Robustness: Robustness was studied out to evaluate the effect of small but deliberate variations in the chromatographic conditions at three different levels, i.e. –2, 0, +2. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the columns with wavelength by ±2 nm (263 nm and 267 nm), mobile phase flow rate by ±2 mL min−1 (0.8 mL min−1 and 1.2 mL min−1), mobile phase pH by ±0.2 units (pH 3.0 and 3.4) had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. The results are shown in **Table: VI**.

3.7 Limit of Detection (LOD) and Limit of Quantification (LOQ): The Limit of Detection (LOD) found was 0.05% and The Limit of Quantification (LOQ) analyzed was 0.15% for PZP. These values reflect the high sensitivity of the method, which is of great importance in most studies and also indicating the method can be used for detection and quantification of analytes in a very wide concentration range.

3.8 Specificity: No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical formulations were tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms.

4. Conclusion

A simple and easily available HPLC method was developed in this study for the quantification of PZP in pharmaceutical matrices. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram

indicates non-interference of the common excipients used in the tablets. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of PZP and can be used for routine analysis in pharmaceutical quality control within a short time.

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Table I: Linear regression data of calibration curves

Parameter	Pazopanib HCl
Concentration range(µg/mL)	20-120
Slope (m)	172694.049
Intercept (Y)	528142.1276
Standard error of estimate (c)	428066.7611
Correlation coefficient (r)	0.998
Linear regression (r^2)	0.997
%RSD	0.4

Table II: Assay & recovery Accuracy studies of pazopanib HCl in tablet dosage forms

Tablet formulation	Amount claim (mg/tablet)	Amount Obtained (mg)* by proposed method	** % Recovery by the Proposed method
	Pazopanib HCl	Pazopanib HCl	Pazopanib HCl
1) 120%	200	203.27	109.57
2) 100%	200	200.05	99.26
3) 80%	200	197.75	92.61
Average Mean	200	200.35	100.48

Average of three determinations** * After spiking the sample**

Accuracy parameter	Pazopanib HCl	
Assay (120%)	129.50%	
Assay (100%)	109.26%	
Assay (80%)	88.22%	
	Standard	Spiked
% RSD (120%)	0.1	0.3
% RSD (100%)	0.5	0.4
% RSD (80%)	0.3	0.3
	Area	
Standard Deviation (120%)	30162.5	70663.3
Standard Deviation (100%)	77817.5	76612.5
Standard Deviation (80%)	35393.9	39949.7

Table: III: Precision of Recommended Procedure Using API- {Pazopanib HCl} (PZP) & its Formulation Matrice (Votrient®)

Sr. No	Inj. No	Name of the Standard Drug & Conc. (200 µg/mL)	Retention time in minutes	Peak Area	Name of the Sample Drug & Conc. (200 µg/mL)	Retention time in minutes	Peak Area
		API (PZP)			Formulation Matrice (Votrient®)		
1	1	PZP	7.417	16963518	Votrient®	7.141	16944572
2	2	PZP	7.358	16939480	Votrient®	7.212	17043405
3	3	PZP	7.281	16941492	Votrient®	7.210	17076312
4	4	PZP	7.136	16932832	Votrient®	7.177	16991681
5	5	PZP	7.199	16894390	Votrient®	7.232	16964333
6	6	PZP	7.142	16930451	Votrient®	7.148	16956173
7	Mean		7.255	16933693.8	Votrient®	7.187	16996079.4
8	Standard Deviation		0.116	22538.3		0.037	52819.9
9	% RSD		1.60	0.1		0.52	0.3

Table IV: Validation Summary / System Suitability

Parameter	Pazopanib HCl (PZP) (Standard API drug)	Votrient® (Sample drug)
Theoretical Plates(N)	4036.35	4140.83
Tailing factor	1.29	1.32
Retention time(min)	7.392	7.386
Resolution	2.55	9.40
Area	17081048	17134315
% Peak Area	99.93	99.93
LOD (µg/mL)	0.05	0.05
LOQ (µg/mL)	0.15	0.15

Table V: Results from testing of the Robustness of the method (n=3, 100% of the Working Standard Solution & Sample Solution contains: 200 µg/mL of Pazopanib HCl (PZP)

Condition Studied in Robustness	Modification In OFAT analysis	Parameter Fixation	Mean Peak Area ± S.D	% RSD (Peak Area)	Mean Retention Time (in min) ± S.D	% RSD (Retention time)
			PZP	PZP	PZP	PZP
Column(s) (XTerra RP18)	Hypersil, & Inertsil ODS C18 3V	Std	16938497.0 ± 13566.3	0.1	7.155 ± 0.051	0.71
		Sample	16949840.7 ± 35836.6	0.2	7.153 ± 0.073	1.03
Flow rate (1.0 ml/min)	1.2 ml/min & 0.8 ml/min	Std – Increase	15839219.6 ± 58320.2	0.4	6.724 ± 0.033	0.49
		Std- Decrease	18966766.3 ± 41198.5	0.2	7.865 ± 0.031	0.40
		Sample- Increase	16107848.3 ± 77066.4	0.5	6.663 ± 0.026	0.40
		Sample- Decrease	18890994.4 ± 70374.5	0.4	7.888 ± 0.024	0.31
pH (3.2)	3.4 & 3.0	Std - Increase	17085698.7 ± 59123.4	0.3	7.691 ± 0.009	0.11
		Std- Decrease	17084204.4 ± 52304.9	0.3	7.072 ± 0.013	0.18
		Sample - Increase	17055489.3 ± 58283.0	0.3	7.677 ± 0.011	0.15
		Sample - Decrease	17053343.7 ± 50867.4	0.3	6.946 ± 0.037	0.53

Figure 4: Calibration Curve of the Pazopanib HCl (PZP) by RP-HPLC

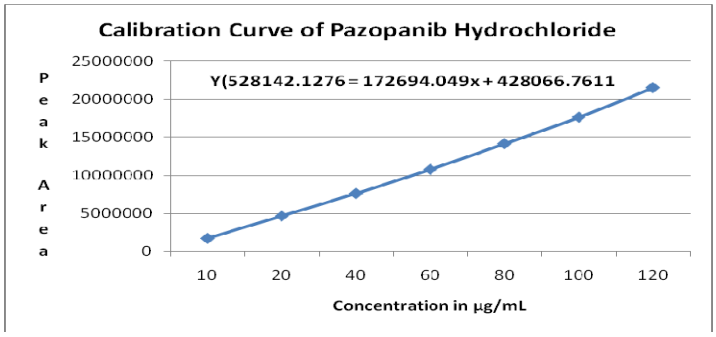
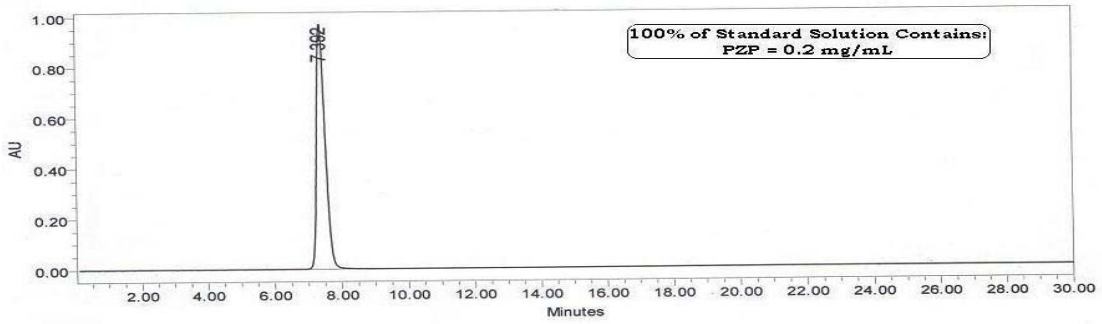
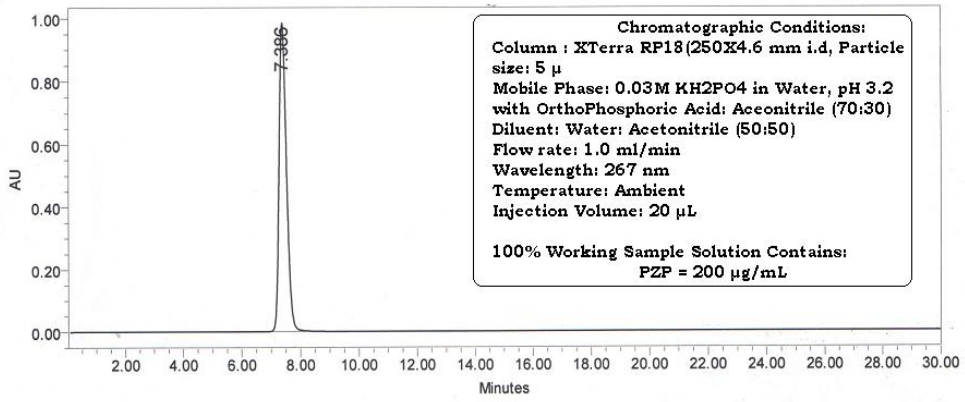


Figure 5 & 6: Typical Chromatogram of Pazopanib HCl (Standard and Working Sample) by RP-HPLC





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