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Research Article

NEW EXTRACTIVE METHOD DEVELOPMENT OF PAZOPANIB HCl IN API AND ITS UNIT DOSAGE FORM BY SPECTROPHOTOMETRY

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ABSTRACT

A simple, economical, precise, reliable and reproducible Extractive Spectrophotometric Method has been developed for the estimation of Pazopanib HCl in bulk as well as in tablet formulations. The developed Method was based on the formation of chloroform extractable complex of Pazopanib HCl, with Bromothymol Blue (BTB) which shows maximum absorption at 414nm. The absorbance-concentration plot is linear over the range 5-50µg/ml.The different experimental parameters effecting the development and stability were studied carefully and optimized. The result of analysis for the method was validated statistically and recovery studies were also performed.

Keywords: Pazopanib HCl, Bromothymol Blue (BTB), Ultraviolet-Visible spectrophotometer.

INTRODUCTION

Pazopanib HCl chemically,5-[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl] amino]-2-methylbenzolsulfonamide.The molecular formula is $C_{21}H_{23}N_7O_2S.HCl$ and molecular weight is 473.99 g/mol.It is a potent and selective multitargeted receptor tyrosine kinase inhibitors of VEGFR-1, VEGFR-2, VEGFR-3 (vascular endothelial growth factor receptor), PDGFR α and β [platelet derived growth factor receptor), C-kit (also known as mast or stem cell growth factor receptor (SCFR) or proto-oncogene c-kit or tyrosine – protein kinase kit or CD117] that blocks tumor growth and inhibit angiogenesis. It has been approved for renal cell carcinoma, soft tissue sarcoma, ovarian cancer and also non-small cell lung carcinoma. The chemical structure of Pazopanib is as shown in figure 1. Till date no methods have been reported in bulk and also in pharmaceutical formulation by using UV –Visible double beam Spectrophotometer. Thus the present study was undertaken to bridge this gap by developing and validating a simple, sensitive, accurate, precise, and reproducible UV spectrophotometric method for estimation of Pazopanib HCl.

MATERIALS AND METHODS

Apparatus

Elico double beam UV –Visible Spectrophotometer SL-244 with 1cm matched quartz cells was used for all spectral measurements.

Reagents

All chemicals used were of analytical reagent grade

- **Bromothymol Blue (BTB) 0.05% w/v** - 0.1 gm of dye sample and dissolve in 10 ml of methanol (95%), add 0.4 ml of 0.02N NaOH and make up to 100 ml with distilled water.

- **Preparation of Phosphate Buffer pH 3.6**: Dissolve 0.900 gm of anhydrous di sodium hydrogen phosphate and 1.298 gm of citric- acid monohydrate in sufficient water to produce 1000 ml.
- Chloroform AR grade.

PROCEDURE

Standard stock solution

A standard stock solution containing 1 mg/ml was prepared by dissolving 100 mg of Pazopanib HCl in 100 ml of methanol. From this, a working standard solution containing 50 µg/ml was prepared with methanol.

Assay procedure

Aliquots of standard drug solution of Pazopanib HCl containing 0.1-1.0 ml (50µg/ml) are taken and transferred into series of separating funnels. To each separating funnel, 2 ml of 0.05% w/v Bromothymol Blue (BTB) and 2 ml of Phosphate buffer pH 3.6 were added. Reaction mixture was shaken gently for 5 min. Then 5ml of chloroform was added to each of them. The contents are shaken thoroughly for 5 min and allowed to stand for 15 minutes, so as to separate the aqueous and chloroform layer. Colored chloroform layer was separated out and absorbance was measured at 414nm against reagent blank. (Absorbance curve was shown in figure 2) Calibration curve was prepared, shown in figure 3. Chemistry of coloured species is shown in figure 4

Preparation of sample solution

12 Tablets of Pazopanib HCl were accurately weighed and powdered. Tablet powder equivalent to 100 mg of Pazopanib HCl was dissolved in 100 ml of methanol and filtered and washed with methanol. The filtrate and washings were combined and the final volume was made to 100 ml with methanol. The solution was suitable diluted and analyzed as given under the assay procedure for bulk samples. The results are represented in table 2. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Pazopanib, by the proposed method.

RECOVERY STUDIES

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The percentage recoveries thus obtained were given in Table 2.

RESULTS AND DISCUSSIONS

In this present work,new method has been developed for the estimation of Pazopanib HCl from tablet formulation. The developed method was based on formation of chloroform extractable colored complex with Bromothymol Blue (BTB).The condition required for formation of colored complex was optimized. Statistical analysis was carried out and the results were satisfactory. Relative standard deviation values were low, that indicates the reproducibility of the proposed methods. Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. The optical characteristics such as absorption maxima, beer's law limit, molar-absorptivity and sandell's sensitivity are presented in table 1. The regression analysis using the method of least square was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in table 1. Assay of Pazopanib HCl, in tablet formulations in table 2.

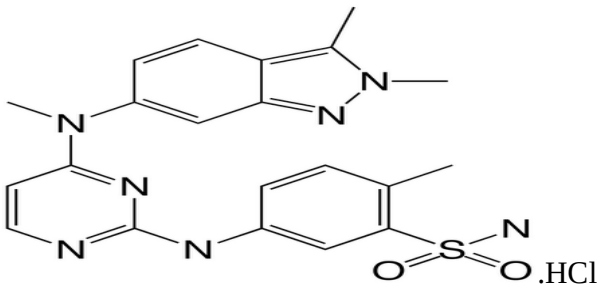

Fig. 1: Chemical structure of Pazopanib HCl

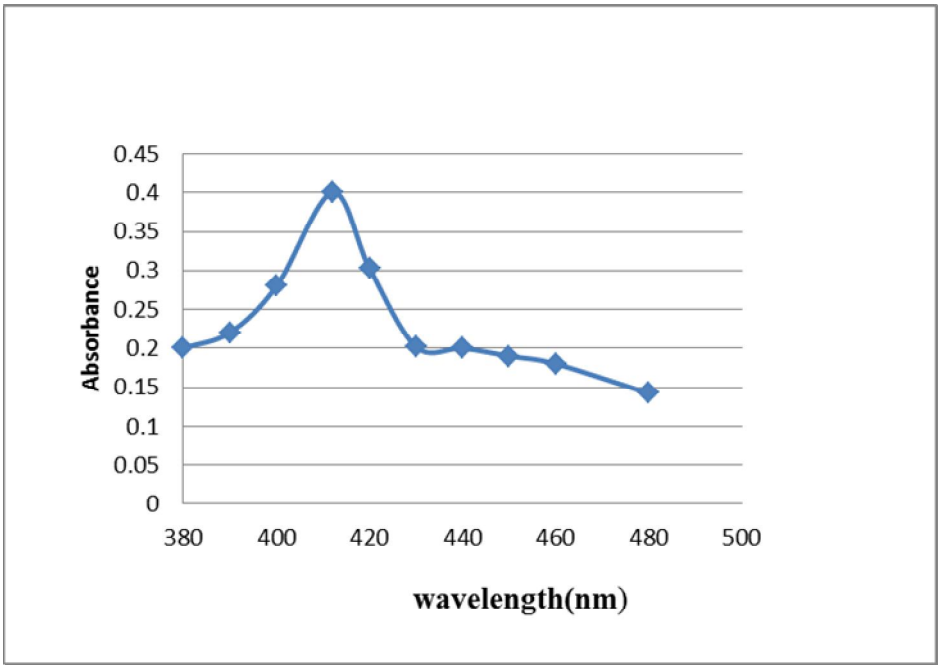

Fig. 2: Absorption spectrum of pazopanib HCl with Bromothmol Blue

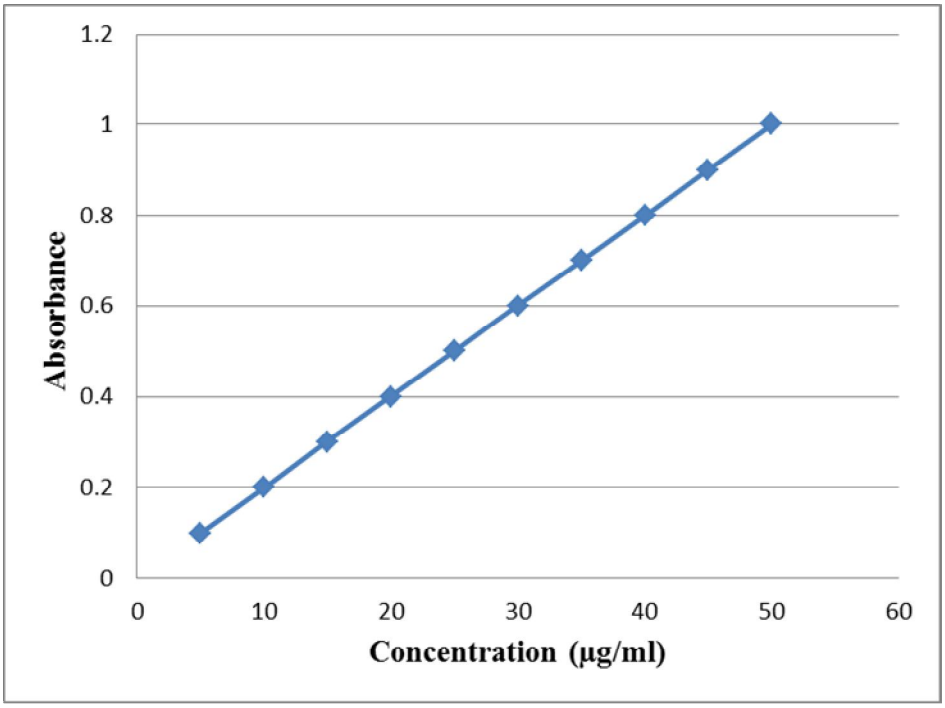

Fig. 3: Standard calibration curve for analysis of pazopanib HCl at 414nm

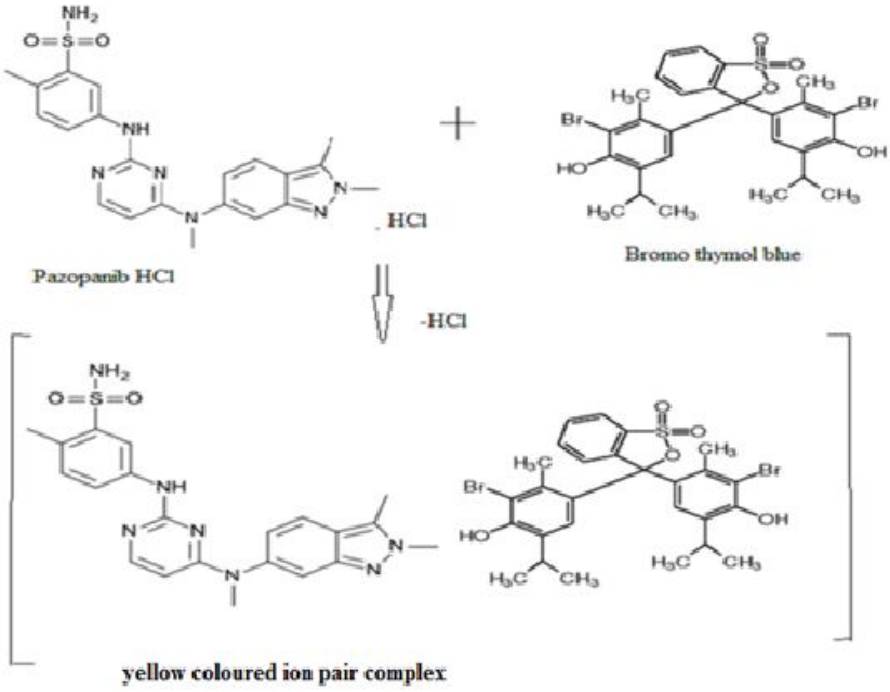

Figure 4: chemistry of coloured species

Table 1: Regression and validation parameters of Pazopanib HCl

Regression parameters		
Sr. No.	**Parameter**	**Result**
1	Slope (m)	0.018
2	Intercept (c)	0.0402
3	% RSD	0.6042
4	Standard Regression Equation	Y=0.018x+0.0402
5	Correlation Coefficient (R)	0.9952
6	Standard error of estimate	0.0294
7	Confidence intervals (upper limit=1)	0.971-0.991
Validation parameters		
1	Absorption maxima(nm)	414
2	Beers law limit (µg/ml)	5-50
3	Molar absorptivity (micrograms/cm2/0.001absorbance unit)	2.2×10^3
4	Sand ell's sensitivity (micrograms/cm2/0.001absorbance unit)	0.6280
5	Limit of Detection (mcg/ml)	0.066
6	Limit of Quantification (mcg/ml)	0.2
7	Optimum photometric range (µg/ml)	5-50
8	Color stability (hours)	1.5
9	Accuracy(% Recovery ±SD)	99.66 ±0.21

Table 2: Assay of Pazopanib HCl in Tablet formulations

Tablet Formulation	Labeled Amount (Mg)	Amount obtained(mg)*By proposed method	%Recovery By the Proposed Method**
1	200	198.92 ± 0.36	99.66 ±0.21
2	200	199.18± 0.21	97.73± 0.11
3	200	199.47± 0.19	98.90± 0.24

*Average of three determinations ** After spiking the sample

CONCLUSION

A spectrophotometric method for quantifying PazopanibHCl in formulation samples has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range studied. In summary, the proposed method can be used for the drug analysis in routine quality control.

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