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Solubility Determination of Pazopanib In Various Lipidic Excipients

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ABSTRACT: Pazopanib (PZB) is an anticancer drug which inhibits multi-tyrosine kinase receptors. It belongs to BCS class II used for the treatment of renal cell carcinoma and soft tissue sarcoma. A simple, rapid, accurate, sensitive and reproducible reverse-phase high-performance liquid chromatographic method (RP-HPLC) has been developed and validated for PZB and the developed method is applied to quantitatively assess the solubility of PZB in various oils, surfactants and cosurfactants. The separation was achieved on a C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column) using a mobile phase composed of 0.1% orthophosphoric acid solution and Acetonitrile in a ratio of 40:60 v/v at a flow rate of 1ml/min. The injection volume of 20µl and the wavelength is 270nm. The retention time of PZB was observed at 4.9 minutes. The method was validated for specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. LOD and LOQ were 0.0374 ng/ml and 0.1133 ng/ml respectively. The calibration curve was linear in the concentration range of 0.2-2 ng/ml with correlation coefficient of 0.9994. The proposed method is validated according to ICH guidelines Q2 (R1). Screening of excipients was done by determining the saturation solubility studies in various oils, surfactants and co-surfactants. Capmul MCM C8 NF selected as an oil phase, Kolliphor RH 40 as surfactant and Transcutol HP as co-surfactant due to their higher solubilization effect.

KEY WORDS: Pazopanib, Oils, RP-HPLC, Retention time, Surfactants, Solubility etc.

I. INTRODUCTION

Pazopanib (PZB) is an anti-cancer drug which is used in treatment of renal cell carcinoma, advanced soft tissue sarcomas and bone sarcoma[1–3]. PZB is a potent and selective multi-targeted receptor tyrosine kinase inhibitor that blocks tumour growth and inhibits angiogenesis[4]. PZB primarily show its inhibitory effect on vascular endothelial growth factor receptor 1, 2 and 3, platelet endothelial growth factor receptor-α, and -β, and the stem-cell factor receptor c-kit[5,6]. It is a BCS class II drug exhibits low aqueous solubility ≤0.33mg/ml with log P value of 3.2 and low oral bioavailability[7,8].

The HPLC method is simple, sensitive and reproducible for PZB determination in various excipients. High Performance Liquid Chromatography which is also known as High Pressure Liquid Chromatography. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture. HPLC is an advanced technique of column liquid chromatography. The solvent usually flows through column with the help of gravity but in HPLC technique the solvent will be forced under high pressures, so that sample can be separated into different constituents with the help of difference in relative affinities[9–12]. Reverse phase liquid chromatographic (RP-HPLC) technique involves separation of components on the basis of its properties and hydrophobicity. The mixture of components was introduced into the mixture of buffer, an organic solvent that acted as the mobile phase. This separation can be accomplished with an elution technique known as isotropic or gradient. The concentration of the mobile phase will be constant throughout the chromatographic process, whereas the composition of the mobile phase will be continuously changed throughout the process[13,14].

The increasing proportion of drug molecules in pharmaceutical development pipelines can be classified under the Biopharmaceutical Classification System (BCS) as a class II compound (compound with high permeability & low solubility). These new drug molecules were discovered and optimized using relatively new technologies. The low solubility of these compounds led to suboptimal patient outcomes due to their low oral bioavailability and variable pharmacokinetics. Simple formulation approaches (conventional tablets or powdered capsules) are not sufficient to overcome these problems[15,16]. Bioavailability enhancing technologies (LBDDS, solid amorphous dispersion, API salting or API particle size reduction) have been developed to solve the problem of low solubility by improving dissolution rates and/or apparent solubility of these drug molecules[17–19]. LBFs are one of the emerging technologies to improve the solubility and bioavailability of poorly soluble drugs. Its solubility can be improved by formulating it in a lipid-based drug delivery system (LBFS). Oils and surfactants are the basic ingredients of the formulation[20–22]. The solubility of PZB was

evaluated in different oils and surfactants by reverse phase high performance liquid chromatography (RP-HPLC). The aim of the study was to develop an accurate, specific, repeatable HPLC method for the determination of pazopanib and to evaluate the best oils and surfactants.

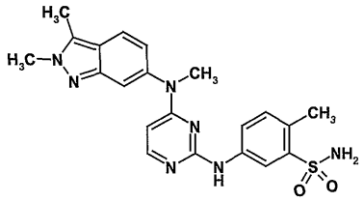

Fig-1: Chemical structure of Pazopanib

II. MATERIALS AND METHODS:

2.1 Materials

Pazopanib was obtained as a gift sample from Aizant drug research solutions pvt ltd. (Hyderabad), Labrafac CC, labrafac PG, labrasol ALF, lauroglycol 90, lauroglycol FCC, peceol, Plurol oleique, labrafil M 1944 CS, capryol 90, masine CC were gift samples from Gattefosse, France., PEG 200, PEG 400, Tween 40, Tween 80 was purchased from Finar reagents., Capmul MCM C8 EP, capmul MCM NF, capmul PG-8 NF, captex 200 are donated from Abitec corporation, USA., Kolliphor HS 15, Kolliphor RH 40 and Kolliphor EL are gift samples from BASF. The water was obtained from Milli-Q-Water purification system, Millipore. Acetonitrile was HPLC Grade Merck and other chemicals were of analytical grade procured from research lab fine chem.

2.2 Methods

2.2.1 Chromatographic system and conditions

The analysis was performed by HPLC Waters 2998 with equipped waters 515 pump and photodiode array detector. Empower software is used for data acquisition. Chromatographic operation performed isocratically at room temperature. The stainless-steel analytical column used for separation was C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column) using a mobile phase composed which is of 0.1% orthophosphoric acid solution and Acetonitrile in a ratio of 40:60 v/v at a flow rate of mobile phase was monitored at 1ml/min and detected at a wavelength 270nm from the scan spectrum shown in figure-2. The injection volume of 10µl with a run time of 10min[23,24].

2.2.2 Method development

The method development was tried with acetonitrile and water in 50:50 v/v ratio, but the results were not satisfactory because of tailing of peaks. Hence, the method is switched to 1% orthophosphoric acid solution and Acetonitrile, the peaks obtained were good and acceptable. Further, to reduce the retention time of peaks the proportion of 0.1% orthophosphoric acid solution: Acetonitrile in a ratio of 40:60 v/v for obtaining shorter retention time. The short method development was done by 3 trials, the conditions maintained in trials are given in Table-1,2 &3. The chromatograms of the trials are shown in Figures-6,7& 8. The trial-3 method is optimized and selected by using the mobile phase: 0.1% orthophosphoric acid solution and Acetonitrile in a ratio of 40:60 v/v, the retention time obtained was 4.9min. The optimized programme for pump A (Acetonitrite) and pump B (0.1% orthophosphoric acid solution) was carried out, and the results were good and reproducible. The absorption maxima was found at 270 nm. The method development trials are discussed in the Table-1, 2 & 3. The column used for all the trials is C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column) and the wavelength of detection is 270nm[11,25].

Table:1 Method development trails

A. Trial-1

Table:1- Method development conditions of Trial-1

Column	SunFire C18 5µm, 4.6×250mm column
Mobile phase	Water and Acetonitrile in a ratio of 50:50 v/v
Diluent	Acetonitrile & water (50:50)
Mode	Isocratic elution
Column temperature	25˚C
Injection volume	20µl

Detection Wavelength	270nm

B. Trial-2

Table:2- Method development conditions of Trial-2

Column	SunFire C18 5µm, 4.6×250mm column
Mobile phase	0.1% orthophosphoric acid solution and Acetonitrile in a ratio of 50:50 v/v
Diluent	Acetonitrile & water (50:50)
Mode	Isocratic elution
Column temperature	25˚C
Injection volume	20µl
Detection Wavelength	270nm

C. Trial-3

Table:3- Method development conditions of Trial-3

Column	SunFire C18 5µm, 4.6×250mm column
Mobile phase	0.1% orthophosphoric acid solution and Acetonitrile in a ratio of 40:60 v/v
Diluent	Acetonitrile & water (50:50)
Mode	Isocratic elution
Column temperature	25˚C
Injection volume	20µl
Detection Wavelength	270nm

2.2.3 Method validation

a) Selectivity and Specificity

The specificity of the method was determined by injecting a blank solution and one of the PZB calibration standards. The ability to respond unambiguous to the analyte in influence of other components. There should be no interference due to blank at the retention time of the PZB, which is shown in Figure-2 & 3.

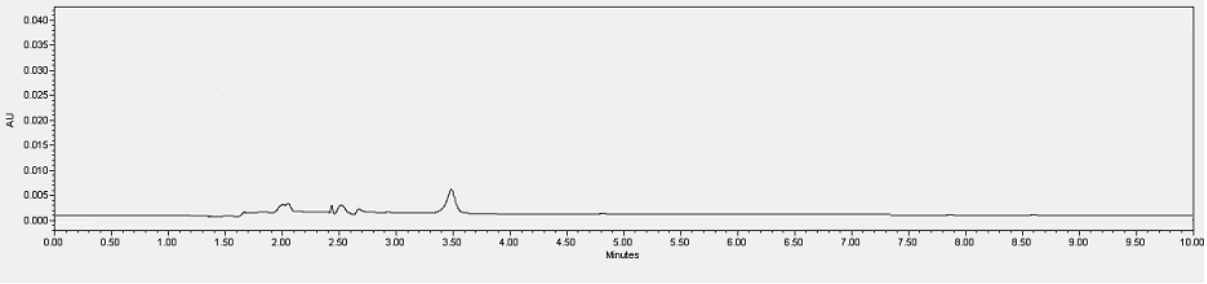

Fig:2 Chromatogram of Blank solution

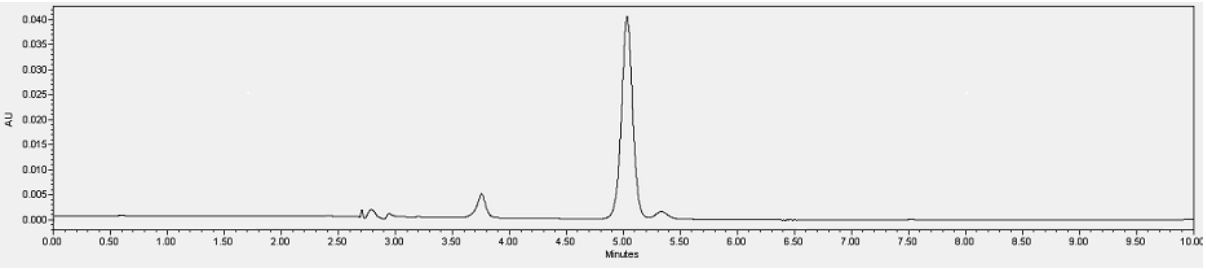

Fig:3 Chromatogram of Pazopanib

b) Linearity and range

A stock solution of 1 mg/ml in acetonitrile was prepared by accurately weighing 100 mg of PZB and dissolving them in 100 ml of acetonitrile. The 100 µg/ml working standard was obtained by diluting the stock solution with acetonitrile and water (50:50). Working standards were diluted to obtain concentrations ranging from 0.2-2 ng/ml that were analysed using the proposed method. The optimized mobile phase ratio was used to construct the calibration curve with the PZB concentration on the x-axis and the peak area on the y-axis[26,27].

Each calibration standard was analysed in triplicate and the slope, intercept and correlation coefficients were calculated. The calibration curve of PZB is shown in Figure-4.

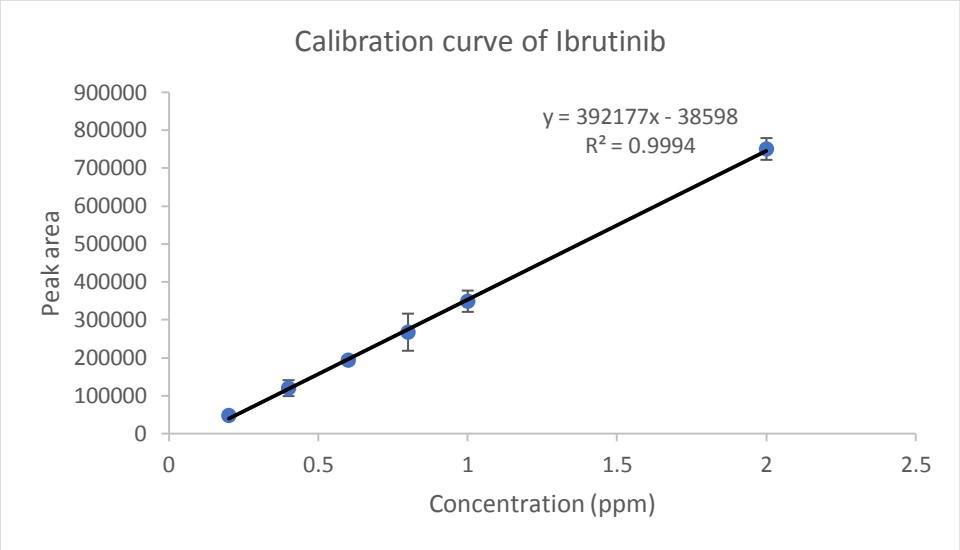

Fig:4 Calibration curve of Pazopanib (n=3)

c) Precision

The precision of the method was determined by intra-day/repeatability precision and inter-day/intermediate precision variation studies. The most important part of any validation study for analytical procedure is precision. The repeatability precision was estimated by analysing the linearity/calibration curves of the six replicates of same concentration of Ibrutinib within the same day. The intermediate precision was assessed by analysing the six replicates of same concentration of PZB on three different days[28,29]. The precision of the method was expressed as %RSD.

d) Accuracy

The accuracy expresses nearness or closeness of the analytical procedure between expected value and value found. To evaluate accuracy, successive analysis of three different concentrations (n=3) are performed by the method developed[9,28]. The mean recovery should be within 90-110%.

e) Limit of detection & Limit of quantification

LOD is the lowest concentration can be estimated but not necessarily quantified under the stated experimental conditions. LOQ is lowest concentration of an analyte that can estimated with acceptable precision and accuracy[11,30,31].

f) Robustness

To determine robustness of the present developed method, the flow rate was studied at 0.8ml/min and 1.2ml/min, effect of the change in wavelength was analysed at 268nm and 272nm and effect of mobile phase composition was assessed at 45:55 and 35:65 v/v of 0.1% orthophosphoric acid solution: Acetonitrile. The percent RSD of robustness trial under these conditions are calculated[27,29].

g) System suitability

System suitability test performed by introducing blank solution one time and standard solution of 100% test solution 6 times into balanced HPLC system. The system suitability parameters are determined[29,32].

2.2.4 Saturation Solubility studies

The solubility of pazopanib was determined by dissolving an excess amount of the drug into 1gm of various oils, surfactants and cosurfactants. The excipients were screened for determining the equilibrium solubility of Pazopanib. The samples in the vials were vortexed for 2min using cyclomixer (REMI CM 101) and kept in shaking incubator with constant shaking for 48 hours at 25˚C for 48h. Addition of drug is continued in unsaturated excipients and cyclo-mixed and kept in shaking incubator (LabTEch). After saturation of the drug in the particular excipient, the equilibrated samples were centrifuged at 3000rpm for 10min[33–35]. The supernatant solution was determined for the concentration of pazopanib using validated HPLC method by using HPLC-Waters with 515 HPLC Pump and SunFire C18 5µm column. The results were expressed graphically in Figures-8, 9 & 10.

III. RESULTS AND DISCUSSION

This method is specific and reproducible for the quantitative determination of PZB in various oils, surfactants and cosurfactants with a short retention time of 4.9min and run time of 10min. The developed method shows shorter retention time compared with other methods entailed in various research papers. The method developed was found to be linear in the range of 0.2-2µg/ml. The developed method is validated as per ICH guidelines. The retention time of the drug PZB in optimized method (Trial 3) was found to 4.9min and chromatogram of drug is compared with blank chromatogram in figures 2 and 3 which indicates specificity of the method. The accuracy of the analytical method was indicated by recovery values from 99.16 - 100.28%. Precision is reflected by %RSD values less than 2. The method was found to robust with variation in wavelength, flow rate and mobile phase composition, the %RSD for all the parameters were found to ≤ 2. The method was successfully applied for estimation of PZB in various oils, Surfactants and Cosurfactants. Pazopanib showed maximum solubility in Capmul MCM C8 NF (36.8 mg/ml) selected as an oil phase, Kolliphor RH 40 (31.6 mg/ml) as surfactant and Transcutol HP (50.3 mg/ml) as co-surfactant.

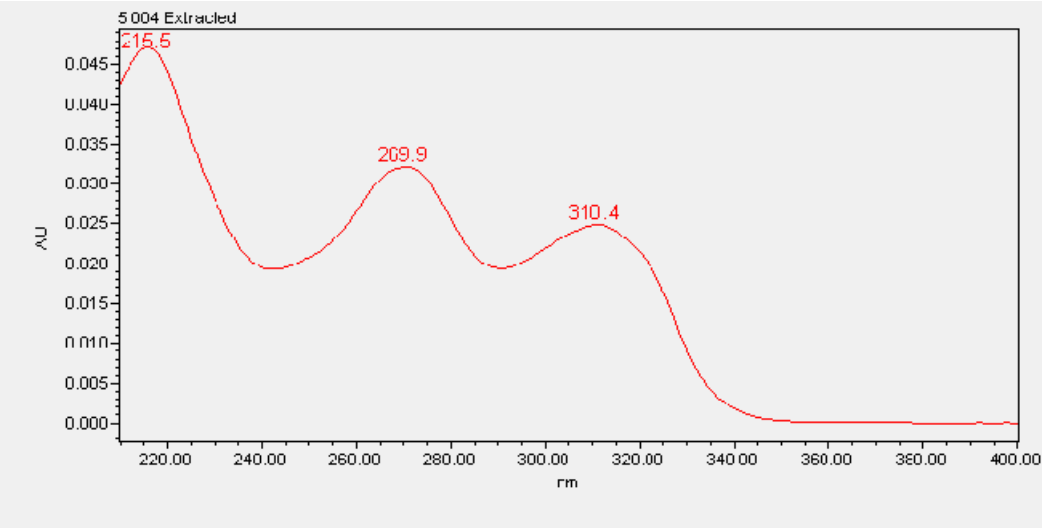

Fig-4: Scan spectrum of Pazopanib by HPLC

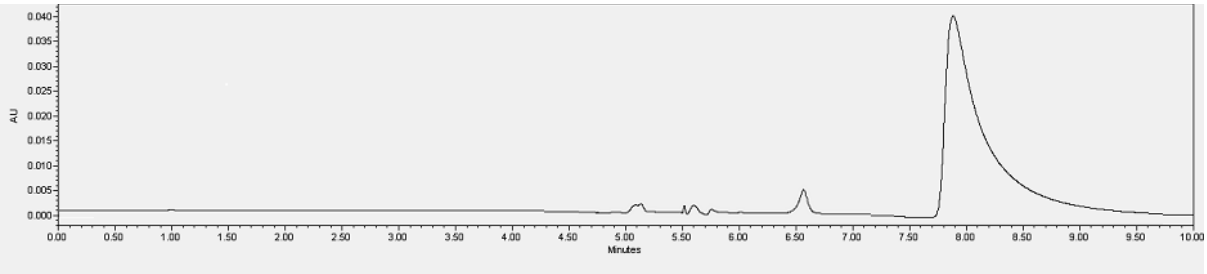

Fig-5: Chromatogram of Trail-1

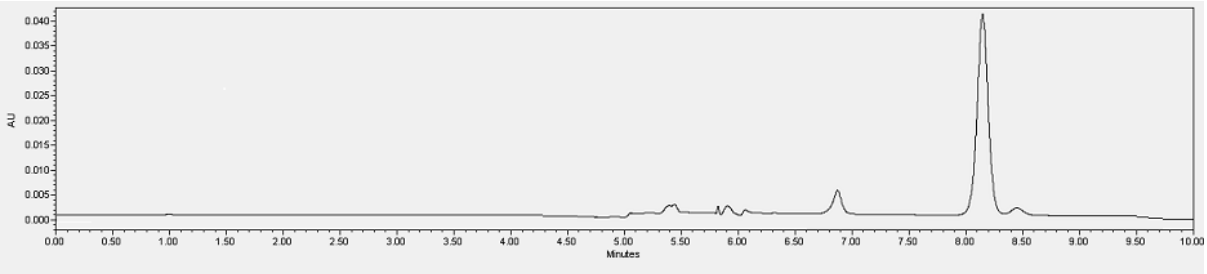

Fig-6: Chromatogram of Trail-2

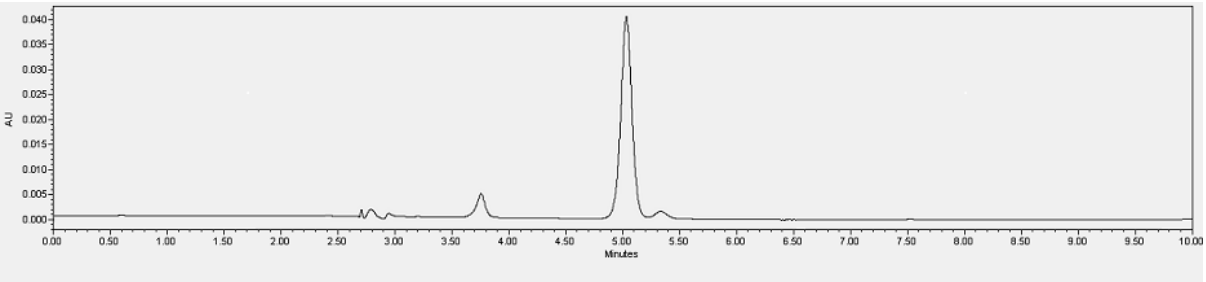

Fig-7: Chromatogram of Trail-3

Table:4 Linear regression data of calibration curve

Concentration range	0.2-2µg/ml
Slope (m)	392177
Y-intercept	38598
Standard error of estimate (c)	4444.89
Correlation coefficient (r^2)	0.9994

Table:4 System suitability

Parameter	**Results**
Retention time (min)	4.9
Tailing factor (USP method)	0.51
Theoretical plates (USP method)	3136
% RSD of peak area	0.96

Table:5 Precision

Precision		**% RSD of 6 replicates**
Intra-day	Retention time	0.72%
	Peak area	0.96%
Inter-day	Retention time	1.23%
	Peak area	0.92%

Table: 6 Accuracy

Test concentration level	**Mean % recovery**
50%	100.28%
100%	99.62%
150%	99.16%

Table:7 Sensitivity

Limit of detection	0.0374 ng/ml
Limit of quantification	0.1133 ng/ml

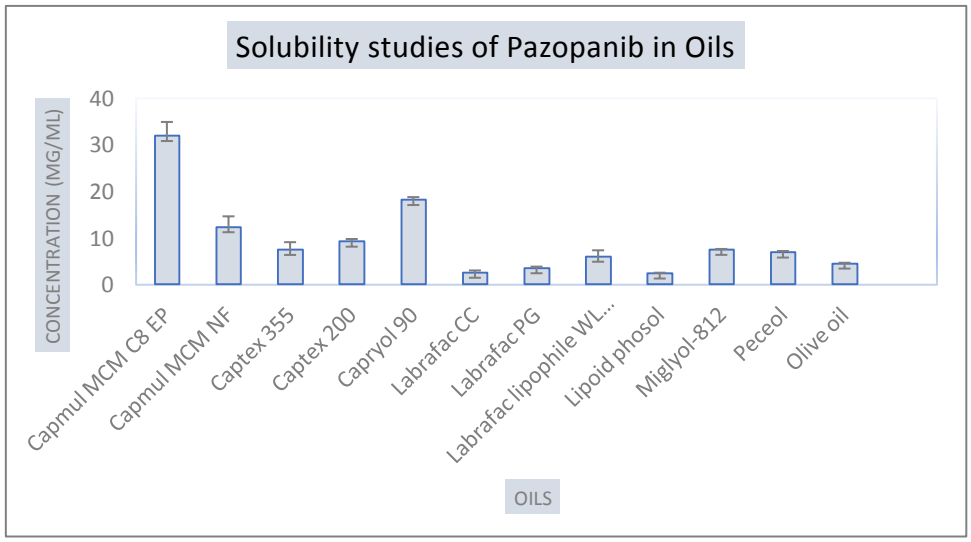

Fig-8: Solubility studies of Pazopanib in various Oils

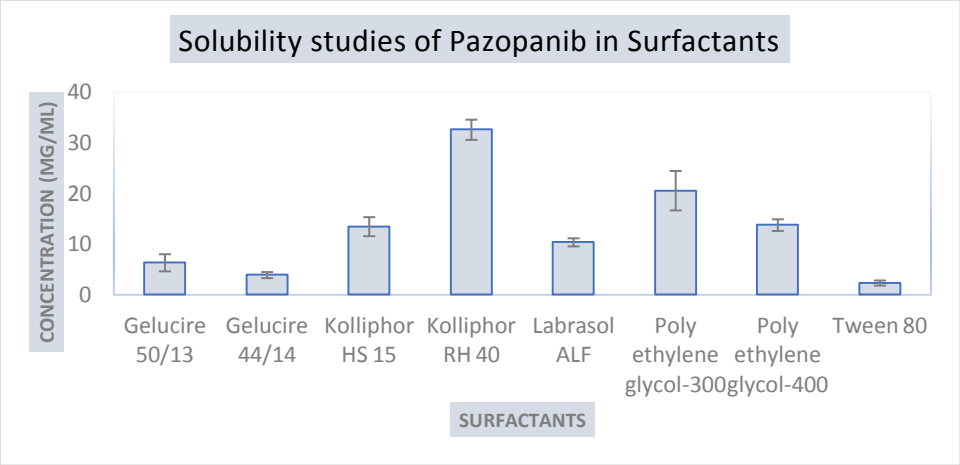

Fig-9: Solubility studies of Pazopanib in various Surfactants

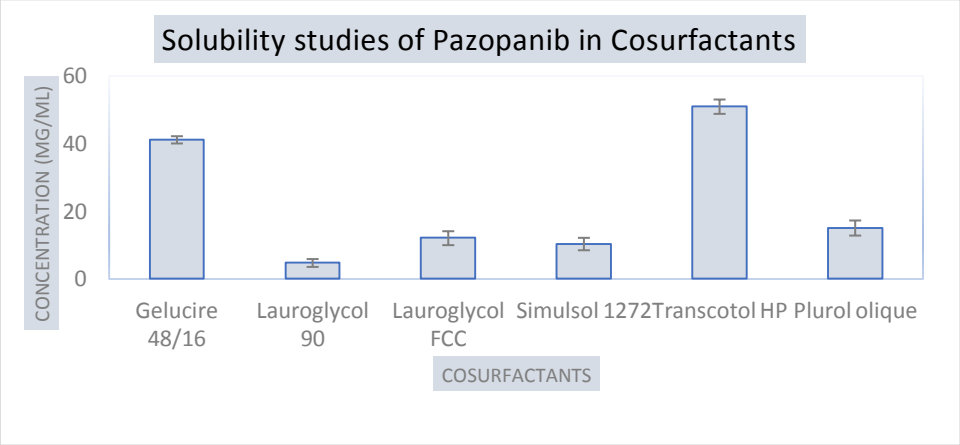

Fig-10: Solubility studies of Pazopanib in various Cosurfactants

IV. CONCLUSION

A new simple, accurate and sensitive RP-HPLC method has been developed for the quantification of Pazopanib and the method was validated and used for quantification of the drug Pazopanib in various oils, surfactants and cosurfactants. The analytical method is precise and accurate with shorter run time of 4.9 min. The solubility studies aimed for identifying suitable oily phase surfactants and cosurfactants for the Pazopanib, Capmul MCM C8 NF selected as an oil phase, Kolliphor RH 40 as surfactant and Transcutol HP as co-surfactant due to their higher solubilization effect.

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