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Screening of Excipients for Lipid Based Drug Delivery Systems of Ibrutinib By Validated HPLC

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Abstract
Ibrutinib is an anticancer drug which binds irreversibly to Bruton's tyrosine kinase (BTK) receptor that binds to a cysteine residue and inhibits BTK active site. Ibrutinib is used for the treatment of mantle cell lymphoma. Mantle cell lymphoma is a fast-growing cancer which begins in the cells of immune system. A new HPLC method has been proposed for the quantification of Ibrutinib. A simple, rapid, accurate, sensitive, reproducible and feasible reverse-phase high-performance liquid chromatographic method (RP-HPLC) has been developed and validated for Ibrutinib and the developed method is applied to quantitatively assess the solubility of Ibrutinib in various oils and surfactants. The separation was achieved on a C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column) using a mobile phase composed of Acetonitrile and 0.1% orthophosphoric acid solution in a ratio of 70:30 v/v at a flow rate of 1ml/min. The injection volume of 10µl and the wavelength is 286nm.The retention time of Ibrutinib was observed at 2.5 minutes. The method was validated for specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. LOD and LOQ were 0.376 µg/ml and 1.128 µg/ml, respectively. The calibration curve was linear in the concentration range of 2-32µg/ml with correlation coefficient of 0.9995. The proposed method is validated according to ICH guidelines Q2 (R1). Ibrutinib showed the highest solubility in Capryol 90 (56.4 mg/ml), Kolliphor EL (39.5 mg/ml) and Transcutol HP (65.7 mg/ml) at 25˚C.

Keywords
Ibrutinib, Oils, RP-HPLC, Retention time, Surfactants, Solubility etc.

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INTRODUCTION
Ibrutinib is an irreversible Bruton's tyrosine kinase (BTK) inhibitor that binds to a cysteine residue in the BTK active site [1]. It is proposed for the treatment of patients with mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) [2]. It blocks the abnormal protein that signals cancer cells multiplication and finally stops dispersion of cancer. Ibrutinib is a small molecule and acts by binding to the protein permanently [3].

The aim of the present work is to develop an accurate, specific, repeatable HPLC method for the determination of Ibrutinib. The HPLC method is simple, sensitive, and reproducible for Ibrutinib 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 [4]. 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[5][6][7]. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters. The proposed method was validated as per ICH guidelines.

An increasing percentage of drug molecules in pharma development pipelines can be classified according to the Biopharmaceutics Classification System (BCS) as Class II compounds (compounds having good permeability but poor solubility) [8]. These newer drug molecules are discovered and optimized through relatively novel technologies. The low solubility and poor permeability of these compounds translates into suboptimal patient outcomes due to poor oral bioavailability and variable pharmacokinetics. Simple formulation approaches (conventional tablets or powder in capsule) are not enough to address these issues. Bioavailability enhancement technologies (LBDDS, solid amorphous dispersions, API salt formation, or API particle size reduction) have been developed to address the issue of low solubility by improving the dissolution rate and/or the apparent solubility of these drug molecules[9][10]. The LBFs are one of the emerging technologies to enhance the solubility and bioavailability of poorly soluble drugs. Its solubility can be enhanced by formulating it into a lipid-based drug delivery system (LBFS). The oils and surfactants are the core ingredients of the formulation [11][12]. The solubility of Ibrutinib is assessed in various oils and surfactants by High performance liquid chromatographic (HPLC)method. The purpose of the study was to assess the best oil and surfactants for the anticancer drug Ibrutinib.

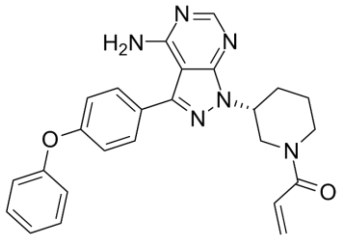

Fig-1: Molecular structure of Ibrutinib

MATERIALS AND METHODS

Materials

Ibrutinib, a gift sample from NACTO Pharma Ltd. Hyderabad., Crodamol PC-LQ, Peceol, Capryol 90, Masine CC, Labrafac PG, Labrafac CC, Labrafil M 1944 CS, Labrasol ALF, Transcutol-HP, Transcutol-P, Miglyol 812, Simulsol 1272, Lauroglycol FCC, Lauroglycol 90, Gelucire-44/14, Gelucire50/13, Gelucire-48/16, Lipoid Phosal 53 were supplied from Gattefosse, France. Capmul MCM C8 EP, Capmul MCM NF, Captex 200, Captex 355 are donated by 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.

Methods

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 Acetonitrile and 0.1% orthophosphoric acid solution in a ratio of 70:30 v/v at a flow rate of mobile phase was monitored at 1ml/min and detected at a wavelength 286nm from the scan spectrum shown in figure-2. The injection volume of 10µl with a run time of 5min. Prior to use the buffer was filtered through Millipore 0.45µm filter and degassed on bath sonicator[13].

Method development

The method development was tried with acetonitrile and water in different proportions, but the results were not satisfactory because of tailing of peaks. Hence, the method is switched to acetonitrile and 1% orthophosphoric acid solution, the peaks obtained were broad and the mobile phase was then changed to acetonitrile and 0.1% orthophosphoric acid solution, the peaks obtained were good and acceptable. Further, to reduce the retention time of peaks the proportion of acetonitrile was increased from 60-70% for obtaining shorter retention time. The method is optimized and selected by using the mobile phase: acetonitrile and 0.1% orthophosphoric acid solution in the ratio of 70:30, the retention time obtained was 2.5min. 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 were found at 286 nm. The method development trials are discussed in the Table-1. 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 286nm [14][15].

Table:1 Method development trails

Optimization condition	Mobile phase	Flow rate	Retention time (Min)	Observation	Result
Trial 1	ACN:Water 50:50	1ml/min	9.5	Tailing of peak	Method rejected
Trial 2	ACN:Water 50:50	0.8 ml/min	6.5	Tailing of peak	Method rejected
Trial 3	ACN:Water 55:45	1ml/min	5.8	Tailing of peak	Method rejected
Trial 4	ACN:Water 60:40	1ml/min	4.6	Tailing of peak	Method rejected
Trial 5	ACN: 1% ortho phosphoric acid 60:40	1ml/min	4.6	Broad peak	Method rejected
Trial 6	ACN: 0.1% ortho phosphoric acid 60:40	1ml/min	4.5	Good peak	Method accepted Further studied for shorter RT
Trial 7	ACN: 0.1% ortho phosphoric acid 65:35	1ml/min	3.4	Good peak	Method Accepted Further studied for shorter RT
Trial 8	ACN: 0.1% ortho phosphoric acid 70:30	1ml/min	2.5	Good peak	Method Accepted

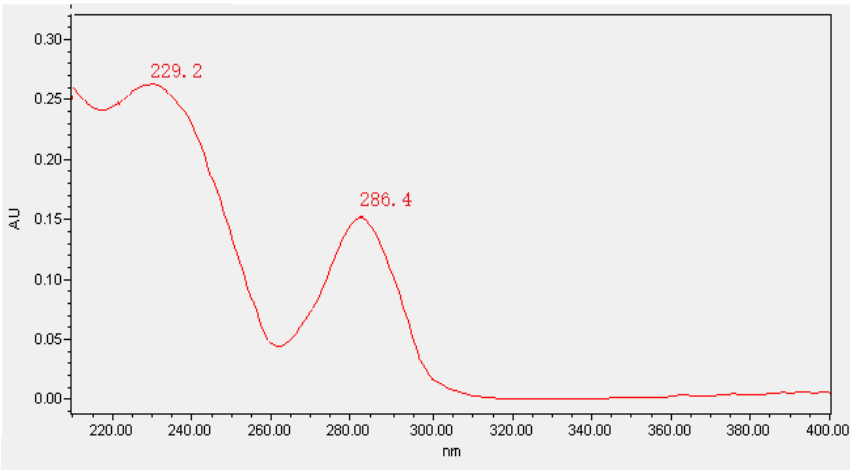

Fig:2 HPLC Scan spectrum of Ibrutinib

Solubility of the drug Ibrutinib in various Oils, Surfactants and Co-surfactants

The solubility of Ibrutinib was studied in various oils, surfactants and cosurfactants. The excess amount of Ibrutinib was added to 1gm of each excipient in cap vial bottle & cyclo-mixed immediately for 5min on cyclomixer (REMI CM 101) and then the resultant mixtures were equilibrated for 72hours on Shaking incubator (LabTech). The supersaturated solutions were centrifuged at a speed of 3000rpm for 15min to remove the undissolved drug. The supernatant was separated, and aliquots of supernatant fluid was drawn by using micro pipette and adequately diluted with Acetonitrile [16][17][18]. The concentration of Ibrutinib in each excipient was quantified by validated RP-HPLC method and graphically represented in Figure-6,7 and 8 by keeping the λ_{max} at 286nm.

Method validation

Selectivity and Specificity

Specificity of the method was determined by injecting the solution of blank and any one of the calibration standards of Ibrutinib. The ability to respond unambiguous to the analyte in influence of other components [19][20][21]. There should be no

interference due to blank at the retention time of the Ibrutinib.

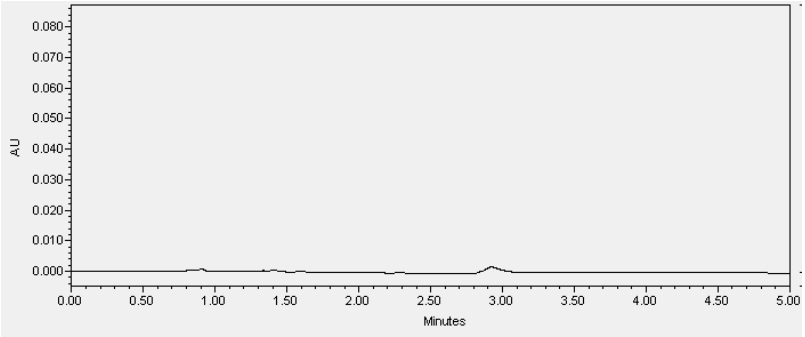

Fig:3 Chromatogram of Blank solution

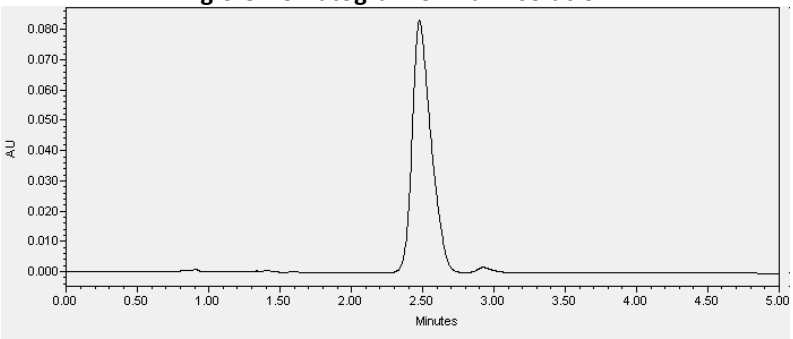

Fig:4 Chromatogram of Ibrutinib

Linearity and range

A stock solution of 1mg/ml in acetonitrile prepared by accurately weighing 100mg of Ibrutinib and dissolving in 100ml of acetonitrile. The working standard of 100µg/ml obtained by diluting the stock solution by acetonitrile. The working standard is diluted to obtain the concentrations ranging from 2-14µg/ml (2, 4, 6, 8, 10, 12 and 14µg/ml) were subjected to analysis by the proposed method. The optimized mobile phase ratio is used for calibration curve construction with concentration of Ibrutinib on x-axis and peak area on y-axis [19][21][20]. Each calibration standard was analyzed three times and the slope, intercept, and correlation co-efficient are calculated.

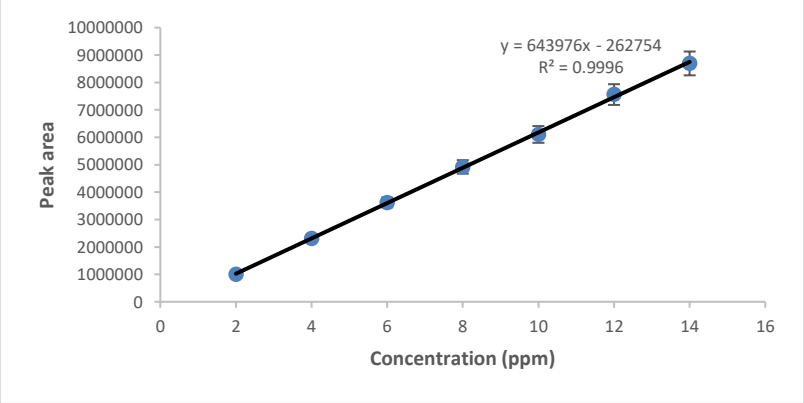

Fig:5 Calibration curve of Ibrutinib (n=3)

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 analyzing the linearity/calibration curves of the six replicates of same concentration of Ibrutinib within the same day. The intermediate precision was assessed by analyzing the six replicates of same concentration of

Ibrutinib on three different days. The precision of the method was expressed as %RSD [19][20][21].

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. The mean recovery should be within 90-110% [19][20][21].

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 [19][20][21].

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 284nm and 288nm and effect of mobile phase composition was assessed at 75:25 and 65:35 of Acetonitrile: 0.1% orthophosphoric acid. The percent RSD of robustness trial under these conditions are calculated [19][20][21].

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 [19][20][21].

RESULTS AND DISCUSSION

This method is specific and reproducible for the quantitative determination of Ibrutinib in various oils, surfactants and cosurfactants with a short retention time of 2.5min and run time of 5min. 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 2-14µg/ml. The developed method is validated as per ICH guidelines. The retention time of the drug Ibrutinib in optimized method (Trial 8) was found to 2.5min and chromatogram of drug is compared with blank chromatogram in figures 3 and 4 which indicates specificity of the method. The accuracy of the analytical method was indicated by recovery values from 99.28 - 100.13%. 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 Ibrutinib in various oils, Surfactants and Cosurfactants. Ibrutinib showed the highest solubility in Capryol 90 (56.4 mg/ml), Kolliphor EL (39.5 mg/ml) and Transcutol HP (65.7 mg/ml).

Table:2 Optimized Chromatographic conditions

Parameter	Optimized condition
Flow rate	1ml/min
Mobile phase composition	Acetonitrile:0.1% orthophosphoric acid solution (70:30)
Diluent	Acetonitrile
Detector Wavelength	286nm
Column	C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column)
Column temperature	Ambient
Injection volume	10µl
Run time	5min

Table:3 Linear regression data of calibration curve

Concentration range	2-14µg/ml
Slope (m)	643976
Y-intercept	262754
Standard error of estimate (c)	53844.67
Correlation coefficient (r^2)	0.9996

Table:4 System suitability

Parameter	Results
Retention time (min)	2.5
Tailing factor (USP method)	0.42
Theoretical plates (USP method)	3235
% RSD of peak area	0.105%

Table:5 Precision

Precision		% RSD of 6 replicates
Intra-day	Retention time	0.63%
	Peak area	0.81%
Inter-day	Retention time	0.98%
	Peak area	0.86%

Table: 6 Accuracy

Test concentration level	Mean % recovery
50%	99.28%
100%	100.13%
150%	99.89%

Table:7 Sensitivity

Limit of detection	0.276 µg/ml
Limit of quantification	0.837 µg/ml

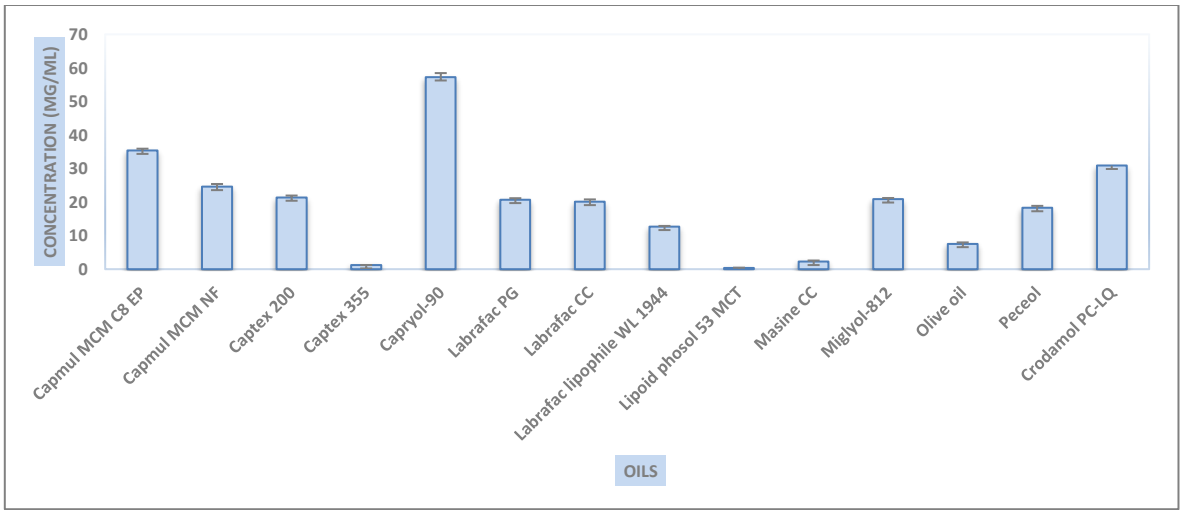

Fig:6 Solubility studies of Ibrutinib in Oils (n=3)

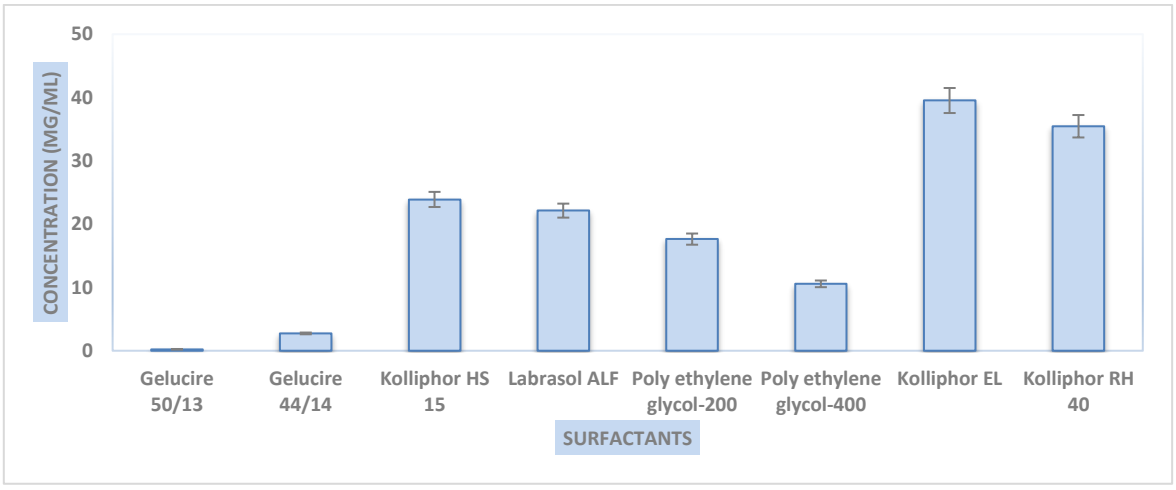

Fig:7 Solubility of Ibrutinib in Surfactants (n=3)

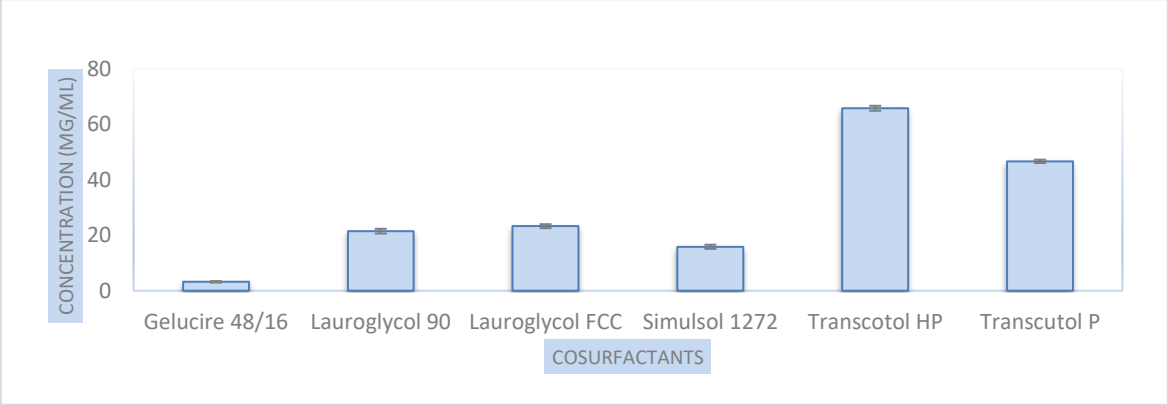

Fig:8 Solubility of Ibrutinib in Cosurfactants (n=3)

CONCLUSION

A new simple, accurate and sensitive RP-HPLC method has been developed for the quantification of Ibrutinib and the method was validated and used for quantification of the drug Ibrutinib in various oils and surfactants. The analytical method is precise and accurate with shorter run time. The solubility studies aimed for identifying suitable oily phase and surfactants for the Ibrutinib to formulate Lipid based drug delivery systems.

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