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Solubility Studies of Gefitinib by Validated High Pressure Liquid Chromatographic Method

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Abstract: **Gefitinib is an anticancer drug which inhibits the intracellular phosphorylation of numerous tyrosine kinase associated with transmembrane cell surface receptors, including tyrosine kinase associated with the epidermal growth factor receptor (EGFR-TK). A simple, rapid, accurate, sensitive, reproducible and feasible reverse-phase high-performance liquid chromatographic method (RP-HPLC) has been developed and validated for Gefitinib and the developed method is applied to quantitatively assess the solubility of Gefitinib 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 6.5pH Phosphate bufferin a ratio of70:30 v/v at a flow rate of 1ml/min. The injection volume of 20µl and the wavelength is 249nm.The retention time of Gefitinib was observed at 4.78 minutes. The method was validated for specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. LOD and LOQ were 0.469 µg/ml and 1.42 µg/ml respectively. The calibration curve was linear in the concentration range of 2-14µg/ml with correlation coefficient of 0.9991. The proposed method is validated according to ICH guidelines Q2 (R1). Gefitinib showed the highest solubility in Peceol (45.4 mg/ml), Labrasol ALF(33.6 mg/ml) and Transcutol P (76mg/ml) at 25˚C.**

Index Terms: **Gefitinib, Oils, RP-HPLC, Retention time, Surfactants, Solubility etc.**

I. INTRODUCTION

Receptor tyrosine kinases (RTK) are key regulatory signalling proteins governing cancer cell growth and metastasis(Nakamura et al., 2005). RTK inhibitors are used in non-small cell lung cancer, breast cancer and other types of cancers(Gschwind et al., 2004),(Lynch et al., 2004).Gefitinib is an inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinases which binds to the ATP binding site of the enzyme(Nakamura et al., 2005). It is a first-generation EGFR inhibitor that shows potential for treatment of various cancers by enhancing the apoptosis of tumour cells and inhibit tumour growth. However, its efficacy is limited due to its poor bioavailability(Hida et al., 2009),(Yanase et al., 2004). Its solubility can be enhanced by formulating it into a lipid-based drug delivery system (LBFS). The LBFs are one of the emerging technologies to enhance the solubility and bioavailability of poorly soluble drugs. The oils and surfactants are the core ingredients of the formulation. (Kalepu et al., 2013),(Pouton& Porter, 2008),(Porter et al., 2007). The solubility of Gefitinib is assessed in various oils and surfactants by High performance liquid chromatographic (HPLC)method(Kazakevich& LoBrutto, 2006). The purpose of the study was to assess the best oil and surfactants for the anticancer drug Gefitinib.

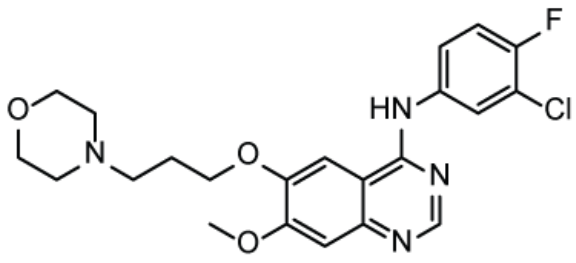

Fig 1: Molecular structure of Gefitinib

II. MATERIALS AND METHODS

A. Materials

Gefitinib, a gift sample from NACTO Pharma Ltd. Hyderabad., Peceol, Capryol 90, Masine CC, Labrafac PG, Labrafac CC, Labrafil M 1944 CS, Labrasol ALF, Transcutol-HP, Transcutol-P, Miglyol 812, Simulsol 1272, LauroglycolFCC, Lauroglycol 90, Gelucire-44/14,

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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 is a gift sample 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.

B. Methods

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 Acetonitrile and 6.5pH Phosphate buffer 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 249nm(Lankheet et al., 2013),(Faivre et al., 2011) from the scan spectrum shown in figure-2. The injection volume of 20µl with a run time of 10min. Prior to use the buffer was filtered through Millipore 0.45µm filter and degassed on bath sonicator(Sabir et al., 2016),(Kazakevich& LoBrutto, 2006).

2) Method development

Different ratios 30:70 to 70:30 of Acetonitrile and pH6.5 phosphate buffer was used as mobile phase and 70:30 of Acetonitrile:pH 6.5 phosphate buffer selected as an appropriate mobile phase which gave a peak with better retention time and acceptable system suitability parameters was chosen for validation and solubility studies(Munir et al., 2014),(D. Gowri sankar et al, 2011)(Sanjay & Kumar, 2012),(Mohd. et al., 2011). The chromatogram of various ratios of mobile phase is shown in Table:1 and figures-3,4 and 5.

Table 1: Method development trails

Optimization condition	Mobile phase-A	Mobile phase-B	Ratio of A/B	Retention Time (min)
Trial 1	ACN	pH 6.5 Phosphate buffer	40:60	8.56
Trial 2	ACN	pH 6.5 Phosphate buffer	50:50	7.73
Trial 3	ACN	pH 6.5 Phosphate buffer	60:40	6.12
Trial 4	ACN	pH 6.5 Phosphate buffer	70:30	4.78

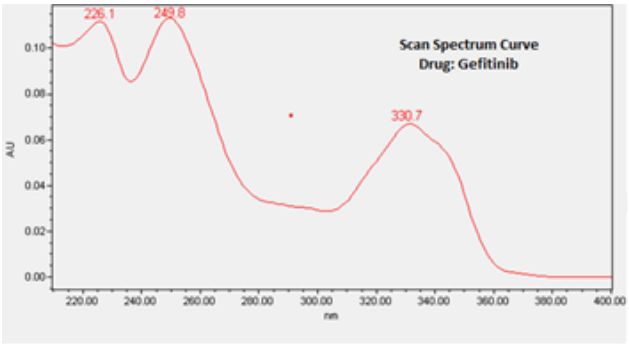


Fig 2: HPLC Scan spectrum of Gefitinib

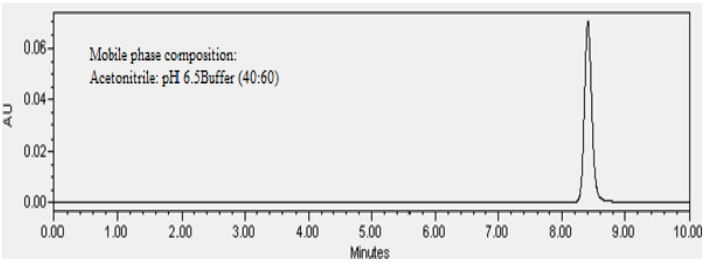


Fig 3: Chromatogram of Trial -1

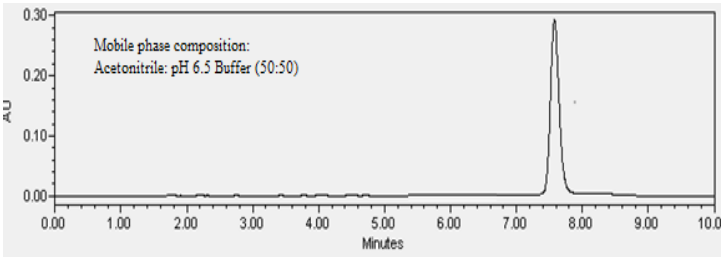
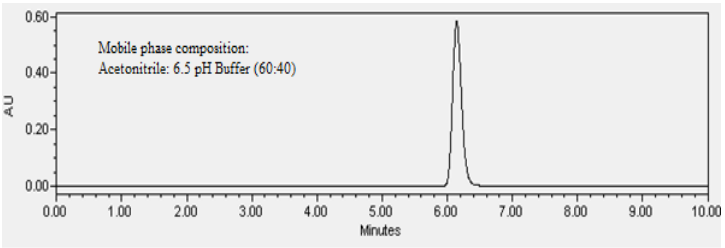


Fig 4: Chromatogram of Trial -2



` Fig 5: Chromatogram of Trial -3

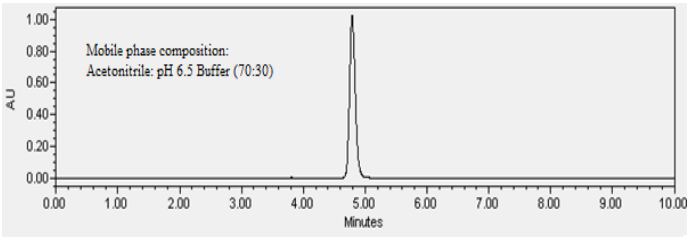


Fig 6: Chromatogram of Trial -4

3) Solubility of the drug Gefitinib in various Oils, Surfactants and Co-surfactants

The solubility of Gefitinib was studied in various oils, surfactants and cosurfactants. The excess amount of Gefitinib 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)(Balakumar et al., 2013),(Singh et al., 2011). 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. The concentration of Gefitinib in each excipient was quantified by validated RP-HPLC method and graphically represented in Figure-10,11 and 12 by keeping the λmax at 249nm(Mohd. et al., 2011),(Dash et al., 2015).

4) Method validation

a) Selectivity and Specificity

Specificity of the method was determined byinjecting the solution of blank and any one of the calibration standard of Gefitinib. 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 Gefitinib (Ali et al., 2011), (Sai Pavan Kumar et al., 2013).

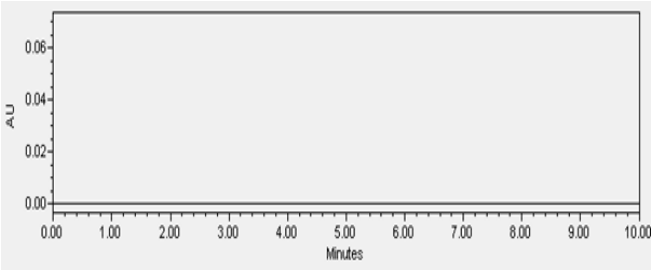


Fig 7: Chromatogram of Blank solution

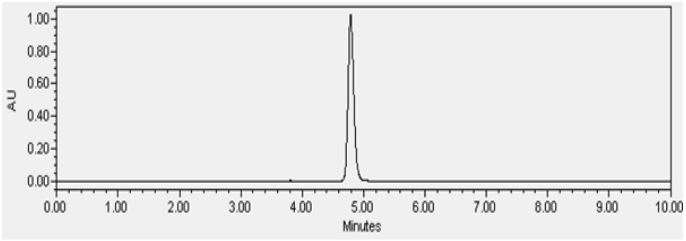


Fig 8: Chromatogram of Gefitinib

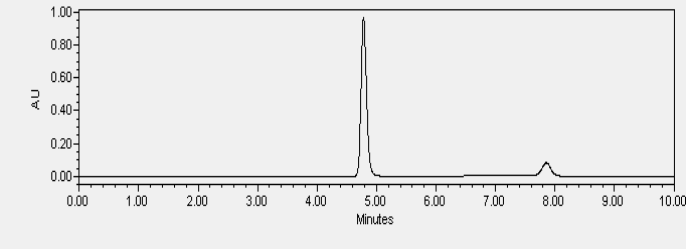


Fig:9 Chromatogram of Gefitinib in presence of Transcutol

b) Linearity and range

A stock solution of 1mg/ml in acetonitrile prepared by accurately weighing 100mg of Gefitinib 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(Gorain et al., 2013),(Munir et al., 2014),(Madishetty& Bontha, 2015). The optimized mobile phase ratio is used for calibration curve construction with concentration of Gefitinib on x-axis and peak area on y-axis. Each calibration standard was analyzed three times and the slope, intercept and correlation co-efficient are calculated.

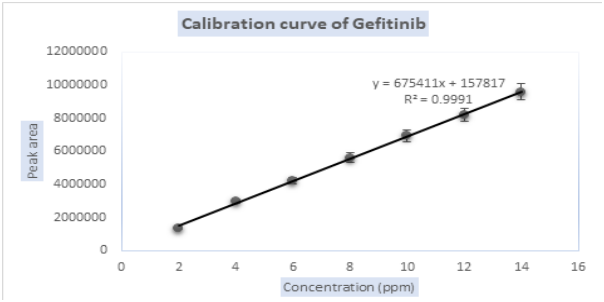


Fig 10: Calibration curve of Gefitinib (n=3)

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 analyzing the linearity/calibration curves of the six replicates of same concentration of Gefitinib within the same day. The intermediate precision was assessed by analyzing the six replicates of same concentration of Gefitinib on three different days. The precision of the method was expressed as %RSD(Bhardwaj et al., 2015).

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. The mean recovery should be within 90-110%(Ibrahim et al., 2019).

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(Munir et al., 2014).

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 247nm and 251nm and effect of mobile phase composition was assessed at 75:25 and 65:35 of Acetonitrile: pH 6.5 phosphate buffer. The percent RSD of robustness trial under these conditions are calculated(Munir et al., 2014).

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(Waghule et al., 2013)

III. Results and Discussion

This method is specific and reproducible for the quantitative determination of Gefitinib in various oils, surfactants and cosurfactants with a short retention time of 4.78min and run time of 10 min. The developed method show shorter retention time compared with other methods entailed in various research papers (D. Peer Basha et al., 2012, Karunakara A 2013). 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 Gefitinib in optimized method (Trial 4) was found to 4.78min and chromatogram of drug is compared with blank chromatogram in figures 7 and 8 which indicates specificity of the method. The accuracy of the analytical method was indicated by recovery values from 99.13 - 100.2%. 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 Gefitinib in various oils, Surfactants and Cosurfactants. Gefitinib showed the highest solubility in Peceol (45.4 mg/ml), Labrasol ALF (33.6 mg/ml) and Transcutol P (76mg/ml)

Table 2: Optimized Chromatographic conditions

Parameter	Optimized condition
Flow rate	1ml/min
Mobile phase composition	Acetonitrile: pH 6.5 Phosphate buffer (70:30)
Diluent	Acetonitrile
Detector Wavelength	249nm
Column	C18-reverse phase column (SunFire C18 5µm, 4.6×250mm column)
Column temperature	Ambient
Injection volume	20µl
Run time	10min

Table 3: Linear regression data of calibration curve

Concentration range	2-14µg/ml
Slope (m)	67541
Y-intercept	157817
Standard error of estimate (c)	95928.73
Correlation coefficient (r^2)	0.9991

Table 4: System suitability

Parameter	Results
Retention time (min)	4.78
Tailing factor (USP method)	0.72
Theoretical plates (USP method)	3289
% RSD of peak area	0.085%

Table 5: Precision

Precision		% RSD of 6 replicates
Intra-day	Retention time	0.56%
	Peak area	0.091%
Inter-day	Retention time	0.74%
	Peak area	0.112%

Table 6: Accuracy

Test concentration level	Mean % recovery
50%	100.2%
100%	99.13%
150%	99.79%

Table 7: Sensitivity

Limit of detection	0.469 µg/ml
Limit of quantification	1.42 µg/ml

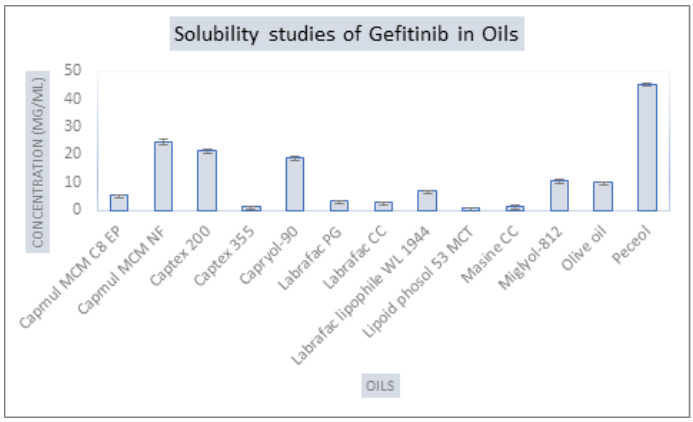


Fig 11: Solubility studies of Gefitinib in Oils (n=3)

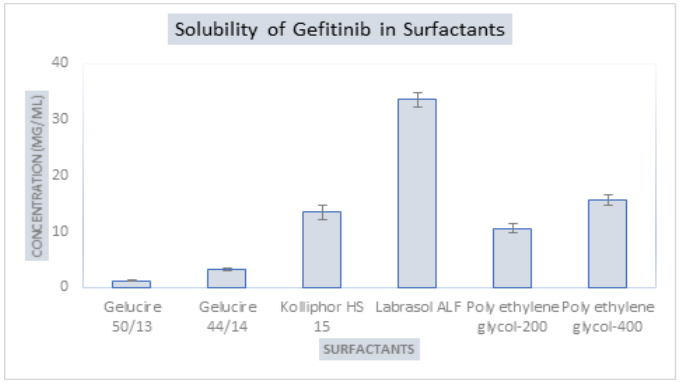


Fig 12: Solubility of Gefitinib in Surfactants (n=3)

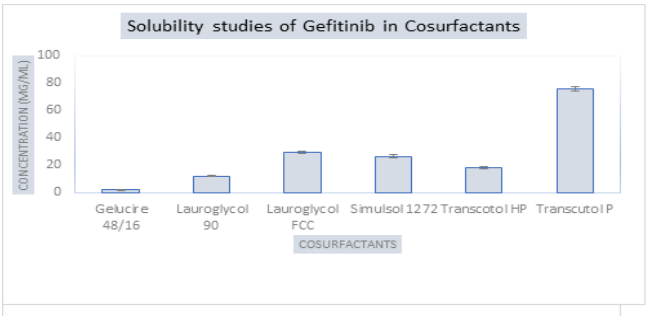


Fig 13: Solubility of Gefitinib in Cosurfactants (n=3)

CONCLUSION

A simple, sensitive and easily available method was developed in this study for quantification of the drug Gefitinib 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 Gefitinib to formulate Lipid based drug delivery systems.

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