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METHOD DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPIC METHOD FOR THE ESTIMATION OF ASSAY OF ANTI CANCER DRUGS- AXITINIB, BOSUTINIB, ERLOTINIB HYDROCHLORIDE, GEFITINIB AND PEMETREXED DISODIUM DRUGS IN API FORM.

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ABSTRACT

The objective of the present work is to develop a simple, efficient and reproducible spectrophotometric method for the quantitative estimation of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drugs in its active pharmaceutical ingredient (API) form. The developed UV-Visible spectrophotometric method for the quantitative estimation of drugs – Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium is based on measurement of absorption at a wavelength maximum (λ_{max}) of 336nm, 269nm, 247nm, 250nm and 225nm using dimethylsulphoxide (DMSO) and methanol as diluents. The method was validated in terms of specificity, precision, linearity, accuracy, and robustness as per the ICH guidelines. The method was found to be linear in the range of 32% to 200% for Bosutinib and Erlotinib hydrochloride drug substances; 40% to 140% for Pemetrexed Disodium drug substance; 50% to 150% for Axitinib and Gefitinib drug substances. The percentage recovery values were in the range of 98.4 to 100.4% for Axitinib, 98.5 to 100.1% for Bosutinib, 99.7 to 100.4% for Erlotinib hydrochloride, 99.7 to 100.5% for Gefitinib and in the range of 99.8 to 100.5% for Pemetrexed Disodium at different concentration levels. Relative standard deviation for precision and intermediate precision results were found to be less than 2%. The correlation co-efficient value observed for linearity of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances was not less than 0.99 for their respective drugs. Results obtained from the validation experiments prove that the developed method is quantified for the estimation of assay of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances. The developed method can be successfully applied for routine analysis, quality control analysis and also suitable for stability analysis of assay of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium in API form as per the regulatory requirements.

KEYWORDS: Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib, Pemetrexed Disodium, Method Development, Validation, UV-Visible spectrophotometry.

INTRODUCTION

Axitinib is a kinase inhibitor. Axitinib has the chemical name N-methyl-2-[3-((E)2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide. The molecular formula is $C_{22}H_{18}N_4OS$ and the molecular weight is 386.47 Daltons. Axitinib is a white to light-yellow powder with a pKa of 4.8. The solubility of axitinib in aqueous media over the range pH 1.1 to pH 7.8 is in excess of 0.2 µg/mL. The partition coefficient (n-octanol/water) is 3.5.[1] Axitinib (AG013736; trade name Inlyta) is a small molecule tyrosine kinase inhibitor developed by Pfizer. It has been shown to significantly inhibit growth of breast cancer in animal (xenograft) models and has shown partial responses in clinical trials with renal cell carcinoma (RCC) and several other tumour types.[2] Soluble in DMSO (42 mg/ml at 25°C), water (<1 mg/ml at 25°C), ethanol (<1 mg/ml at 25°C), methanol, and DMF (~0.25 mg/ml).[3] Axitinib structure is shown in Fig. 1.

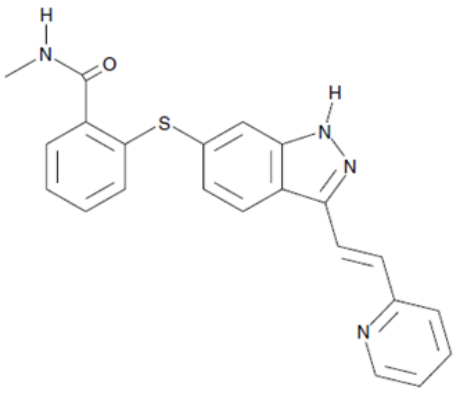

Fig: 1. Structure of Axitinib

Bosutinib (rINN/USAN; codenamed SKI-606, marketed under the trade name Bosulif) is a tyrosine kinase inhibitor undergoing research for use in the treatment of cancer. Originally synthesized by Wyeth, it is being developed by Pfizer. Bosutinib received US FDA and EU European Medicines Agency approval on September 4, 2012 and 27 March 2013 respectively for the treatment of adult patients with Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) with resistance, or intolerance to prior therapy.[4]

Bosutinib is a kinase inhibitor. The chemical name for bosutinib monohydrate is 3-Quinolinecarbonitrile, 4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl) propoxy]-, hydrate (1:1). Its chemical formula is $C_{26}H_{29}Cl_2N_5O_3 \cdot H_2O$ (monohydrate); its molecular weight is 548.46 (monohydrate), equivalent to 530.46 (anhydrous). Bosutinib monohydrate is a white to yellowish-tan powder. Bosutinib monohydrate has a pH dependent solubility across the physiological pH range. At or below pH 5, bosutinib monohydrate behaves as a highly soluble compound. Above pH 5, the solubility of bosutinib monohydrate reduces rapidly.[5] Bosutinib monohydrate structure is shown in Fig. 2.

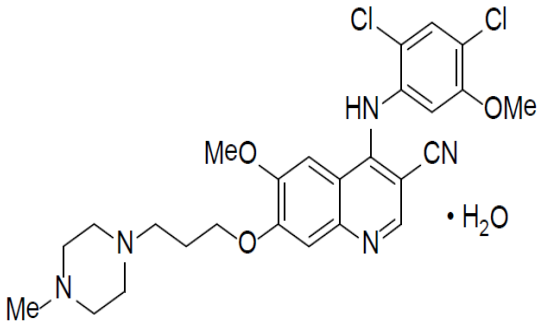

Fig: 2. Structure of Bosutinib

Erlotinib hydrochloride (trade name Tarceva) is a drug used to treat non-small cell lung cancer (NSCLC), pancreatic cancer and several other types of cancer. It is a receptor tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR). Erlotinib has shown a survival benefit in the treatment of lung cancer in phase III trials. The SATURN (Sequential Tarceva in Unresectable NSCLC) study found that erlotinib added to chemotherapy improved overall survival by 19%, and improved progression-free survival (PFS) by 29%, when compared to chemotherapy alone. The U.S. Food and Drug Administration (FDA) has approved erlotinib for the treatment of locally advanced or metastatic non-small cell lung cancer that has failed at least one prior chemotherapy regimen. In November 2005, the FDA approved erlotinib in combination with gemcitabine for treatment of locally advanced, unresectable, or metastatic pancreatic cancer. In lung cancer, erlotinib has been shown to be effective in patients with or without EGFR mutations, but appears to be more effective in patients with EGFR mutations. Overall survival, progression-free survival and one-year survival are similar to standard second-line therapy (docetaxel or Pemetrexed Disodium). Overall response rate is about 50% better than standard second-line chemotherapy. Patients who are non-smokers, and light former smokers, with adenocarcinoma or subtypes like BAC are more likely to have EGFR mutations, but mutations can occur in all types of patients. A test for the EGFR mutation in cancer patients has been developed by Genzyme.[6]

Erlotinib is a quinazolinamine with the chemical name N-(3-ethynylphenyl)-6,7bis(2-methoxyethoxy)-4-quinazolinamine. Erlotinib hydrochloride has the molecular formula $C_{22}H_{23}N_3O_4.HCl$ and a molecular weight of 429.90. The molecule has a pKa of 5.42 at 25°C. Erlotinib hydrochloride is very slightly soluble in water, slightly soluble in methanol and practically insoluble in acetonitrile, acetone, ethyl acetate and hexane. Aqueous solubility of erlotinib hydrochloride is dependent on pH with increased solubility at a pH of less than 5 due to protonation of the secondary amine. Over the pH range of 1.4 to 9.6, maximal solubility of approximately 0.4 mg/mL occurs at a pH of approximately 2.[7] Erlotinib hydrochloride structure is shown in Fig. 3.

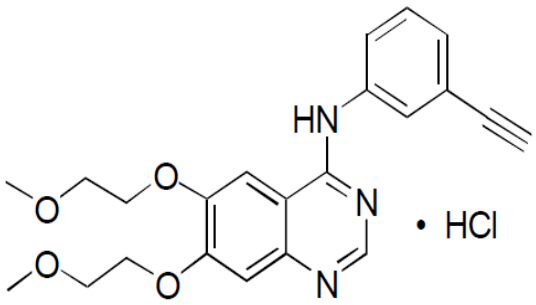

Fig. 3. Structure of Erlotinib hydrochloride

Gefitinib (ZD1839) (Trade name Iressa, marketed by AstraZeneca and Teva), is a drug used for certain breast, lung and other cancers. Gefitinib is an EGFR inhibitor, like erlotinib, which interrupts signaling through the epidermal growth factor receptor (EGFR) in target cells. Therefore, it is only effective in cancers with mutated and overactive EGFR. However, meta-analyses have not shown any improvement in overall survival, that is, it does not extend life. The FDA approved Gefitinib in May 2003 for non-small cell lung cancer (NSCLC). It was approved as monotherapy for the treatment of patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapies i.e. as a third-line therapy. Iressa was approved and marketed from July 2002 in Japan, making it the first country to import the drug. In Europe gefitinib is indicated since 2009 in advanced NSCLC in all lines of treatment for patients harbouring EGFR mutations. This label was granted after gefitinib demonstrated as a first line treatment to significantly improve progression-free survival vs. a platinum doublet regime in patients

harbouring such mutations. IPASS has been the first of four phase III trials to have confirmed gefitinib superiority in this patient population. In most of the other countries where gefitinib is currently marketed it is approved for patients with advanced NSCLC who had received at least one previous chemotherapy regime. However, applications to expand its label as a first line treatment in patients harbouring EGFR mutations is currently in process based on the latest scientific evidence. In 2014 in the TRANSCOG study (Petty et al.), demonstrated gefitinib was effective in esophageal cancer patients whose tumours harboured additional copies of the EGFR gene. Erlotinib is another EGFR tyrosine kinase inhibitor that has a similar mechanism of action to gefitinib. On July 13, 2015, the FDA approved gefitinib as a first-line treatment for NSCLC.[8]

The chemical name of gefitinib is N-(3-Chloro-4-fluorophenyl)-7-methoxy-6-[3-(morpholin-4-yl) propoxy] quinazolin-4-amine. Gefitinib has the molecular formula $C_{22}H_{24}ClFN_4O_3$, a relative molecular mass of 446.9 daltons and is a white-colored powder. Gefitinib is a free base. The molecule has pKa's of 5.4 and 7.2. Gefitinib can be defined as sparingly soluble at pH 1, but is practically insoluble above pH 7, with the solubility decreasing sharply between pH 4 and pH 6. In non-aqueous solvents, gefitinib is freely soluble in glacial acetic acid and dimethyl sulfoxide, soluble in pyridine, sparingly soluble in tetrahydrofuran and slightly soluble in methanol, ethanol (99.5%), ethyl acetate, propan-2-ol and acetonitrile[9] Gefitinib structure is shown in Fig. 4.

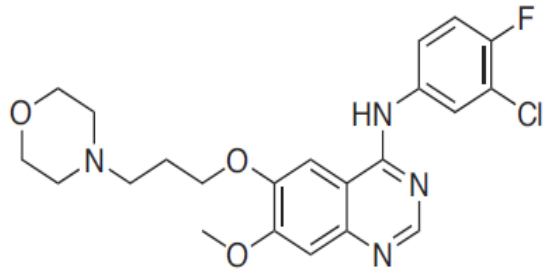

Fig. 4. Structure of Gefitinib

Pemetrexed Disodium (brand name Alimta) is a chemotherapy drug manufactured and marketed by Eli Lilly and Company. Its indications are the treatment of pleural mesothelioma and non-small cell lung cancer. In February 2004, the Food and Drug Administration approved Pemetrexed Disodium for treatment of malignant pleural mesothelioma, a type of tumor of the lining of the lung, in combination with cisplatin for patients whose disease is either unresectable or who are not otherwise candidates for curative surgery. In September 2008, the FDA granted approval as a first-line treatment, in combination with cisplatin, against locally advanced and metastatic non-small cell lung cancer (NSCLC) in patients with non-squamous histology. A Phase III study showed benefits of maintenance use of Pemetrexed Disodium for non-squamous NSCLC. Activity has been shown in malignant peritoneal mesothelioma. Trials are currently testing it against esophagus and other cancers.[10]

The chemical name of the active substance Pemetrexed disodium (heptahydrate) is L-Glutamic acid, N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-disodium salt, corresponding to the molecular formula $C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ and has a relative molecular mass 597.49 g/mol. It appears as a white to almost-white, amorphous, non-hygroscopic crystalline powder. It is freely soluble in water, 0.01N HCl, Formate Buffer, pH 4.0, 0.1N NaOH, very slightly soluble in 0.1N HCl and ethanol, and soluble in methanol.[11] Pemetrexed Disodium structure is shown in Fig. 5.

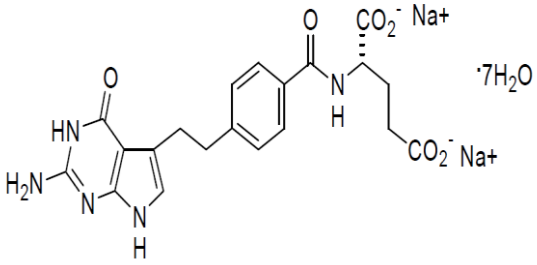

Fig. 5. Structure of Pemetrexed Disodium

From the literature survey it is evident that very few research articles are available for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and for Pemetrexed Disodium drug substances. Chandra reddy et al. published an article on Development and validation of stability indicating RP-HPLC method for the determination of Axitinib in Bulk and its Pharmaceutical Formulations.[12] Lakshmi et al published an article on RP-HPLC method development and validation for the analysis of Axitinib in pharmaceutical dosage forms.[13] Jadhav et al published an article on development and validation of an RP-HPLC method for Bosutinib in bulk form.[14] Kalyana CV et al published an article on development and validation of RP-HPLC method for estimation of Erlotinib in bulk and its pharmaceutical formulation.[15] Faivre et al published an article on a simple HPLC-UV method for the simultaneous quantification of gefitinib and erlotinib in human plasma.[16] Saravanan et al published an article on a stability indicating LC assay method for Pemetrexed Disodium.[17]

Analytical methods are not available in USP[18] and Ph. Eur.[19] for the quantitative determination of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drugs. The present research work describes the estimation of assay content of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium in active pharmaceutical ingredient form by using UV-Visible spectrophotometry technique. Developed method gives a sensitive, specific, and economical method for the determination of

Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium in very short time by using UV-Visible spectrophotometer. Dimethylsulfoxide for Axitinib and methanol for Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium were used in diluent preparation based on the drug solubility properties of their respective drugs. Developed UV-Visible spectrophotometric method was validated with respect to specificity, linearity, precision, accuracy, and robustness parameters.

EXPERIMENTAL

Materials and Methods

Qualified standards (Axitinib ~99.9%, Bosutinib~99.9%, Erlotinib hydrochloride~100.0%, Gefitinib~99.8%, Pemetrexed Disodium ~99.9%) and samples are obtained from Spectrum pharma research solutions and were used without any further purification. HPLC grade methanol (MeOH purity ~99.8%) from Rankem (India) and dimethylsulfoxide (DMSO purity ~99.9%) from Qualigens were used for analytical testing of standard and sample solutions.

Instrumentation

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for the recording of spectra and measuring absorbance for method development and validation study.

METHOD DEVELOPMENT

Selection of Diluent

Initial trails were performed with methanol, water and methanol: water (1:1) to dissolve the Axitinib drug substance and the resultant solutions were not clear from all these diluents. Axitinib drug substance is easily dissolved in Dimethylsulfoxide with clear solution. Dimethylsulfoxide was used as diluent for the preparation of standards and samples of Axitinib and methanol was used as diluent for the preparation of standards and samples of Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances based on the solubility characteristics of the drug substances.

Selection of suitable wavelength detection

Spectra for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium were measured from 200 to 800 nm for wavelength maxima by recording UV-Visible spectrum of standard solution. The corresponding spectrum of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium are shown in Fig.8, Fig.10, Fig.12, Fig.14 and Fig.16. Maximum absorbance (λ_{max}) was shown at 336nm, 269nm, 247nm, 250nm and 225nm for standard solution of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium respectively. Based on the spectra maxima-336nm, 269nm, 247nm, 250nm and 225nm were selected for identification and quantification of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances.

Preparation of standard and sample solutions for Axitinib

Standard stock solution of Axitinib

Accurately weighed and transferred 50 mg of Axitinib working standard into a 50 mL volumetric flask. Added about 30 mL of DMSO (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well.

Preparation of standard solution

Transferred 0.4 mL of standard stock solution of Axitinib into a 50 mL volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution

Accurately weighed and transferred 50 mg of Axitinib drug substance into a 50 mL volumetric flask. Added about 30 mL of DMSO (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well. Further, diluted 0.4 mL of Axitinib sample stock solution into a 50 mL volumetric flask and diluted up to the mark with the diluent and mixed well. Transferred the resultant sample solution into UV cuvettes for analysis.

Preparation of standard and sample solutions for Bosutinib

Standard stock solution of Bosutinib

Accurately weighed and transferred 50 mg of Bosutinib working standard into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well.

Preparation of standard solution

Transferred 0.2 mL of standard stock solution of Bosutinib into a 50mL volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution

Accurately weighed and transferred 50 mg of Bosutinib drug substance into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well. Further, diluted 0.2 mL of Bosutinib sample stock solution into a 50 mL volumetric flask and diluted up to the mark with the diluent and mixed well. Transferred the resultant sample solution into UV cuvettes for analysis.

Preparation of standard and sample solutions for Erlotinib hydrochloride

Standard stock solution of Erlotinib hydrochloride

Accurately weighed and transferred 50 mg of Erlotinib hydrochloride working standard into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and

sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well.

Preparation of standard solution
Transferred 0.2 mL of standard stock solution of Erlotinib hydrochloride into a 50mL volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution
Accurately weighed and transferred 50 mg of Erlotinib hydrochloride drug substance into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well. Further, diluted 0.2 mL of Erlotinib hydrochloride sample stock solution into a 50 mL volumetric flask and diluted up to the mark with the diluent and mixed well. Transferred the resultant sample solution into UV cuvettes for analysis.

Preparation of standard and sample solutions for Gefitinib
Standard stock solution of Gefitinib
Accurately weighed and transferred 50 mg of Gefitinib working standard into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well.

Preparation of standard solution
Transferred 0.4 mL of standard stock solution of Gefitinib into a 50mL volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution
Accurately weighed and transferred 50 mg of Gefitinib drug substance into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well. Further, diluted 0.4 mL of Gefitinib sample stock solution into a 50 mL volumetric flask and diluted up to the mark with the diluent and mixed well. Transferred the resultant sample solution into UV cuvettes for analysis.

Preparation of standard and sample solutions for Pemetrexed Disodium
Standard stock solution of Pemetrexed Disodium
Accurately weighed and transferred 50 mg of Pemetrexed Disodium working standard into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well.

Preparation of standard solution
Transferred 0.5 mL of standard stock solution of Pemetrexed Disodium into a 50mL volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution
Accurately weighed and transferred 50 mg of Pemetrexed Disodium drug substance into a 50 mL volumetric flask. Added about 30 mL of methanol (diluent) and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well. Further, diluted 0.5 mL of Pemetrexed Disodium sample stock solution into a 50 mL volumetric flask and diluted up to the mark with the diluent and mixed well. Transferred the resultant sample solution into UV cuvettes for analysis.

METHOD VALIDATION

Specificity/stress studies
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.[20] The specificity of the developed method was established to prove the absence of interference from diluent absorbance which is a part of required pharmaceutical drug substance preparation.

Linearity
Linearity is the ability of the method to obtain results which are either directly, or after mathematical transformation proportional to the concentration of the analyte within a given range. The linearity of response for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium were determined in the range from 50% to 150% for Axitinib and Gefitinib drug substances; 40% to 140% for Pemetrexed Disodium drug substance; 32% to 200% for Erlotinib hydrochloride and Bosutinib drug substances. The five concentrations of each component were subjected to regression analysis by least squares method to calculate correlation co-efficient and calibration equation. The method of linear regression was used for the data evaluation.

Precision
Precision is a measure of the reproducibility of the whole analytical method under normal operating conditions. The precision was expressed as the relative standard deviation (RSD).
% RSD = (Standard deviation/ average) x 100 Precision of the developed method was carried out by 6 determinations (preparations) of the test solution by measuring the absorbance of test solution and calculated the % RSD for estimation of drug content.

Accuracy
Accuracy or trueness was determined by applying the method to samples in which known amounts of analyte have been added. These should be analyzed against standard and blank solutions to ensure that no

interference exists. The accuracy was calculated from the test results as a percentage of the analyte recovered by the assay.

Accuracy of the present method was carried out by using the drug substance spiked solution at three different concentration levels of 50%, 100% and 150% for Axitinib and Gefitinib; 40%, 100% and 140% for Pemetrexed Disodium drug substance; 32%, 100% and 200% levels for Erlotinib hydrochloride and Bosutinib drug substances in triplicate determinations. Percent recovery and the mean percentage recovery was calculated for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances.

Robustness

Robustness of the method indicates the reliability of an analysis to assess the system suitability parameters under the influence of small but deliberate variations in method parameters. Robustness was performed by changing the detection wavelength ± 2nm to the wavelength maximum (λ_{max}) and calculating the %assay and %RSD for the test solution.

Solution stability

Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium sample solutions and the standard solutions were prepared as per the test procedure. All these solutions were divided into two portions. One portion was stored at room temperature and the other portion was stored in the refrigerator at 2-8°C. Freshly prepared solutions and the solutions which were stored at room temperature and in refrigerator (2-8°C) up to 24 hours were measured for absorbance at different time intervals. The % assay obtained at initial was compared with the % assay obtained at different time intervals.

RESULTS AND DISCUSSION

Optimization of UV-Visible spectrophotometric method conditions

The main purpose of the current method is to develop a simple, sensitive and precise UV-Visible spectrophotometric method for the estimation of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium for the routine quantitative determination of samples which will reduce tedious sample preparations, cost of materials and man power required to perform the analysis. Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium are UV-absorbing molecules with specific chromophores in the structure that absorb at a particular wavelength and this absorbance was successfully employed for their quantitative determinations using the UV spectroscopic method. The spectral analysis showed that the λ_{max} for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium are at 336nm, 269nm, 247nm, 250nm and 225nm respectively. Dimethylsulfoxide was selected as diluent for the standard and sample solutions of Axitinib and methanol was selected as diluent for the standard and sample solutions of Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances. Thus the developed UV -Visible Spectroscopic method for the analysis of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium in its active pharmaceutical ingredient form enables analysis of several samples at the same time due to its simplicity in performing the analysis.

The UV-Vis spectra of Blank run for Dimethylsulfoxide, Blank run for methanol, Axitinib standard solution (concentration -8 µg mL^{-1}), Axitinib sample solution (concentration -8 µg mL^{-1}), Bosutinib standard solution (concentration -4 µg mL^{-1}), Bosutinib sample solution (concentration -4 µg mL^{-1}), Erlotinib hydrochloride standard solution (concentration -4 µg mL^{-1}), Erlotinib hydrochloride sample solution (concentration -4 µg mL^{-1}), Gefitinib standard solution (concentration -8 µg mL^{-1}), Gefitinib sample solution (concentration -8 µg mL^{-1}), Pemetrexed Disodium standard solution (concentration -10 µg mL^{-1}), Pemetrexed Disodium sample solution (concentration -10 µg mL^{-1}) are shown in Fig. 6- Fig. 17.

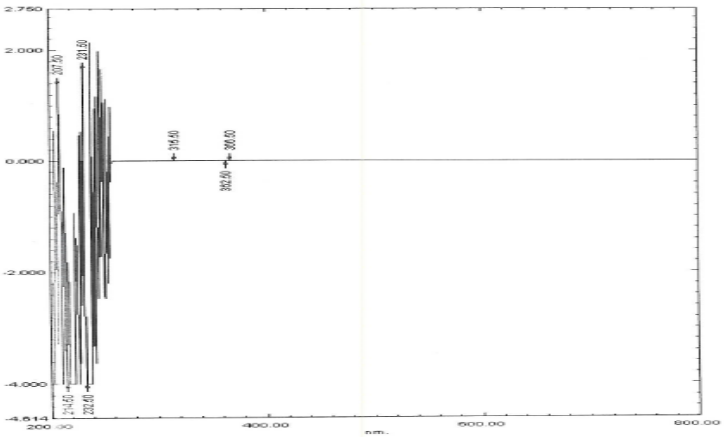

Fig. 6: Blank Spectrum of Dimethylsulfoxide (DMSO)

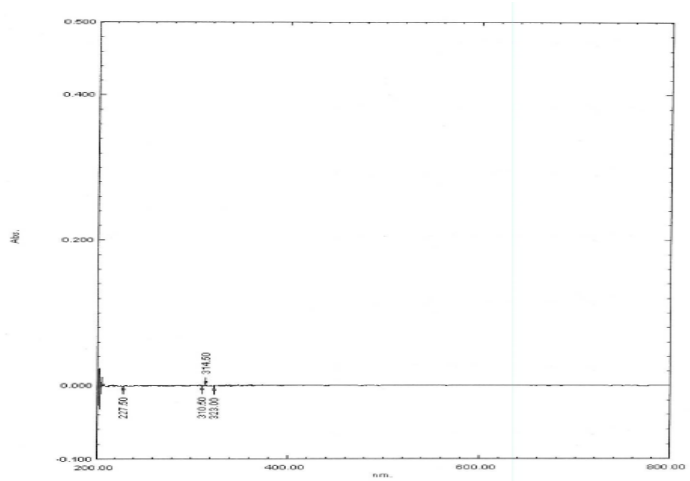

Fig. 7: Blank Spectrum of Methanol

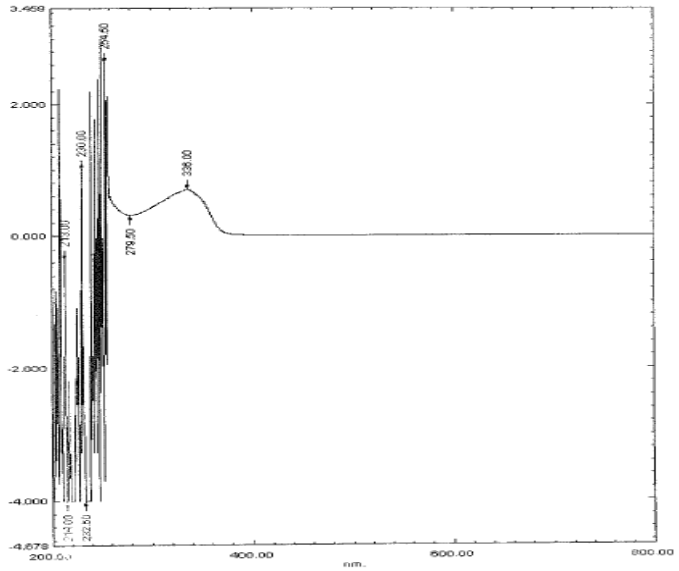

Fig. 8: Axitinib Standard Spectrum

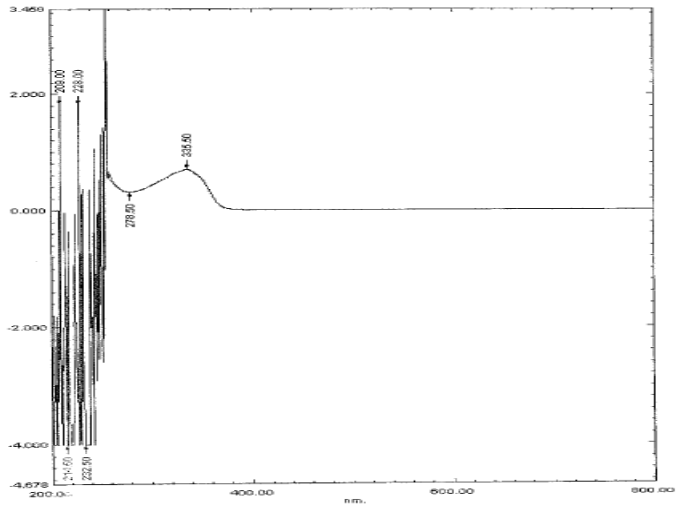

Fig. 9: Axitinib Sample Spectrum

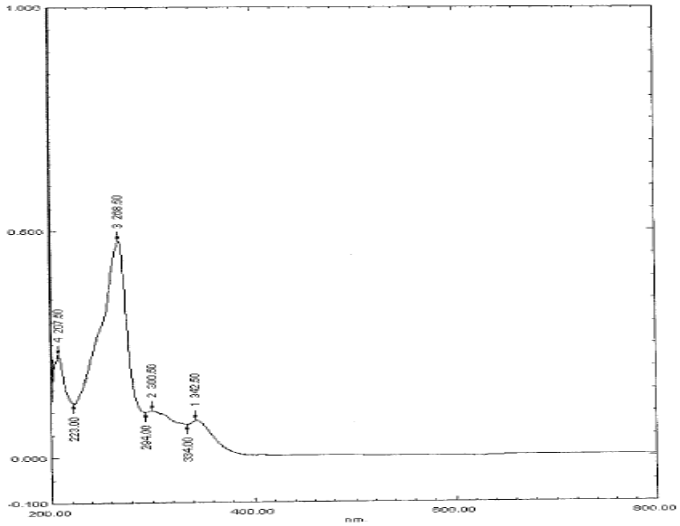

Fig. 10: Bosutinib Standard Spectrum

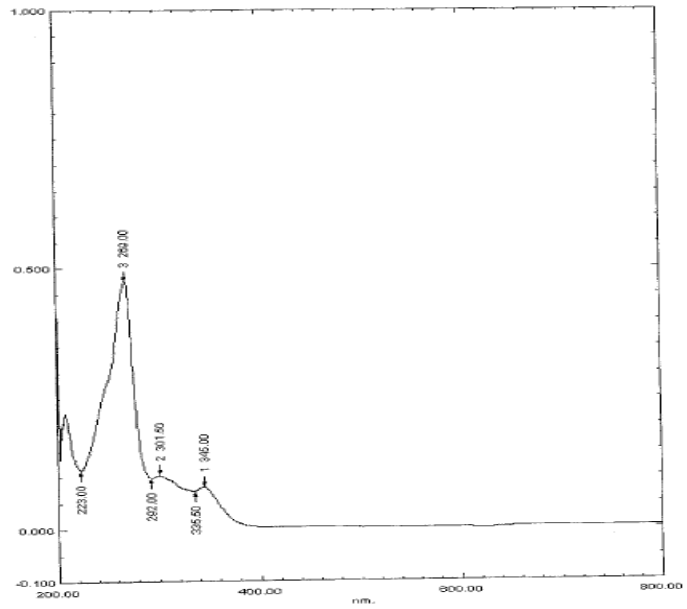

Fig. 11: Bosutinib Sample Spectrum

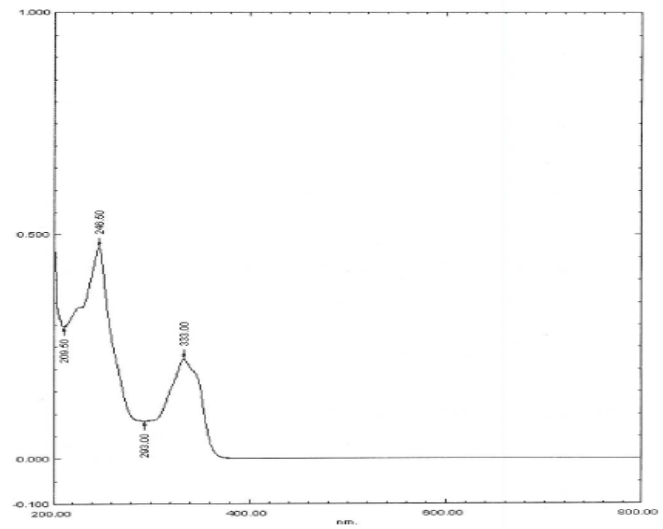

Fig. 12: Erlotinib hydrochloride Standard Spectrum

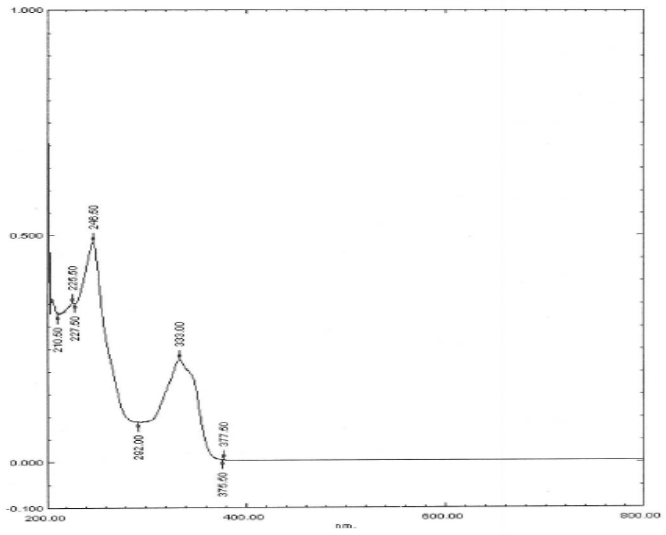

Fig. 13: Erlotinib hydrochloride Sample Spectrum

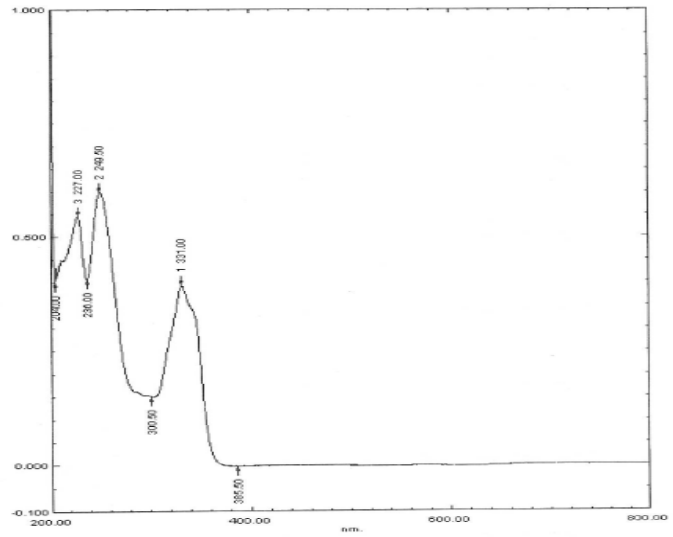

Fig. 14: Gefitinib Standard Spectrum

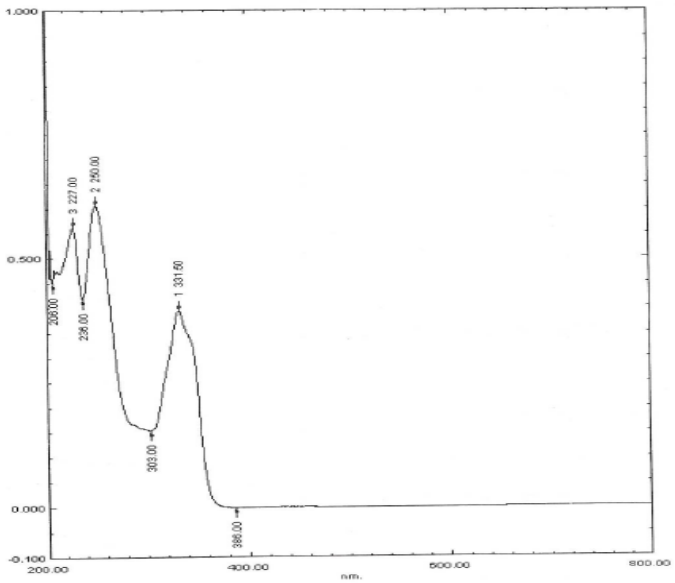

Fig. 15: Gefitinib Sample Spectrum

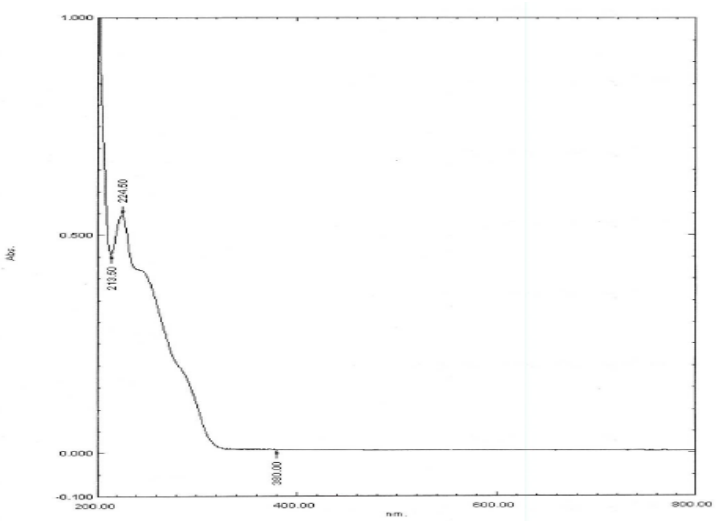

Fig. 16: Pemetrexed Disodium Standard Spectrum

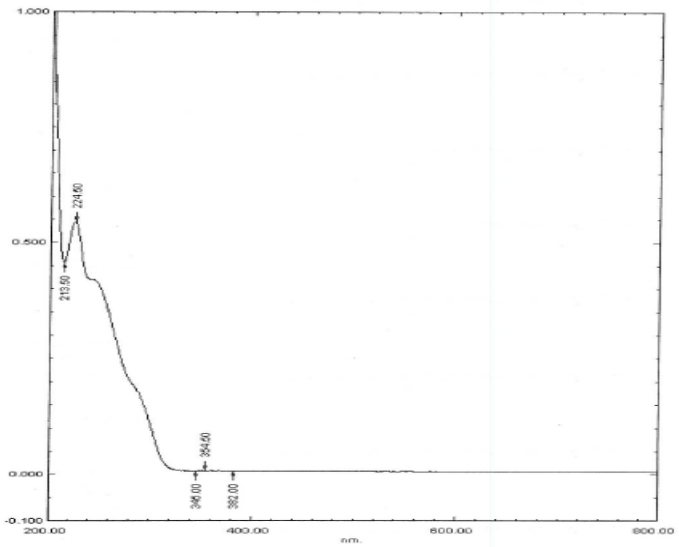

Fig. 17: Pemetrexed Disodium Sample Spectrum

METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended use. The described UV-Visible spectrophotometric method for the estimation of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium has been extensively validated for identification and quantification of its drug substances as per ICH guidelines.[20] After successful completion of method development.[21-22], method validation[20,23-25] was performed to ensure that the developed method was capable of giving reproducible and reliable results when used by different operators employed on the same equipment of the same laboratory or of different laboratories. The developed UV-Visible spectrophotometric method was validated to quantify Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium in its active pharmaceutical ingredient form by determining the parameters including specificity, precision, linearity, accuracy and robustness according to the ICH guidelines.

Specificity

Specificity of the developed method was performed by scanning the UV-Visible spectra of diluent, standard and sample solutions of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium from 200 to 800 nm. Also spectral homogeneity of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium control samples found to be similar with those obtained for the standard solutions of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium.

Precision

Method precision was determined by analyzing the test solution of six determinations and the observed values of % RSD were shown in Table 1 and Table 2. %RSD for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium compounds in test solution for six determinations was not more than 2.0%. Intermediate precision of the method was studied by analyzing the test solution of six determinations and the observed results

were shown in Table 1 and Table 2. The %RSD difference between the two analysts is 0.0%, 0.0%,0.0%, 0.1% and 0.0% for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium respectively. Less difference between the two analysts shows that the developed method is precise and has good intermediate precision.

Table 1: Precision and Intermediate precision data of Axitinib, Bosutinib and Gefitinib

Determination	For Axitinib		For Bosutinib		For Gefitinib	
	Method Precision (% Assay)	Intermediate Precision (% Assay)	Method Precision (% Assay)	Intermediate Precision (% Assay)	Method Precision (% Assay)	Intermediate Precision (% Assay)
Determination-1	99.8	99.8	99.8	99.8	100.0	99.6
Determination-2	99.7	99.7	99.6	99.7	99.8	100.1
Determination-3	99.7	100.0	99.5	99.8	99.9	99.5
Determination-4	99.8	99.8	99.4	100.1	99.8	99.7
Determination-5	99.7	99.7	99.6	99.8	99.6	99.6
Determination-6	99.5	99.7	99.7	99.9	100.0	99.5
Average	99.69	99.80	99.60	99.85	99.85	99.68
SD	0.09	0.12	0.12	0.13	0.14	0.21
%RSD	0.09	0.12	0.12	0.13	0.14	0.21

Table 2: Precision and Intermediate precision data of Erlotinib hydrochloride and Pemetrexed Disodium

Determination	For Erlotinib hydrochloride		For Pemetrexed Disodium	
	Method Precision (% Assay)	Intermediate Precision (% Assay)	Method Precision (% Assay)	Intermediate Precision (% Assay)
Determination-1	99.9	99.9	100.0	100.2
Determination-2	99.9	100.0	99.7	99.9
Determination-3	99.7	100.1	99.9	100.0
Determination-4	99.7	99.9	99.7	99.9
Determination-5	99.9	99.8	99.6	99.6
Determination-6	100.1	99.9	100.2	99.9
Average	99.86	99.94	99.84	99.90
SD	0.14	0.13	0.21	0.19
%RSD	0.14	0.13	0.21	0.19

Linearity

The linearity graphs were plotted between the absorbance versus concentration to obtain the calibration curve. Linearity graphs for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium were shown in Fig.18- Fig.22. The response obtained for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium was found to be linear in the range of 50% to 150% for Axitinib and Gefitinib drug substances; 40% to 140% for Pemetrexed Disodium drug substance; 32% to 200% for Erlotinib hydrochloride and Bosutinib drug substances. The correlation co-efficient observed for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium compounds was not less than 0.99 and also statistical values of all compounds were shown in Table 3. Linearity results demonstrate that an excellent correlation between the absorbance and concentration of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances.

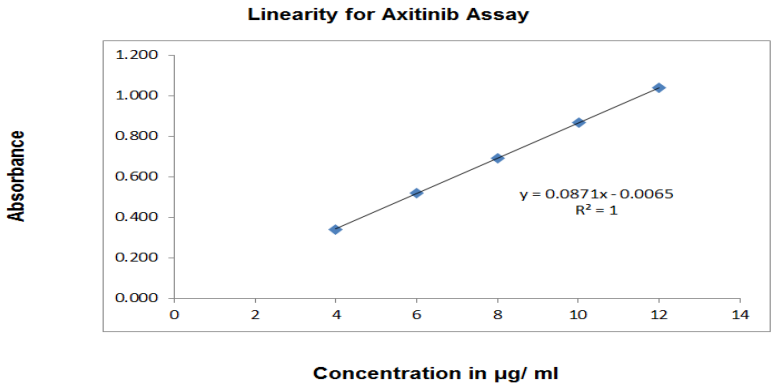

Fig. 18: Linearity graph of Axitinib

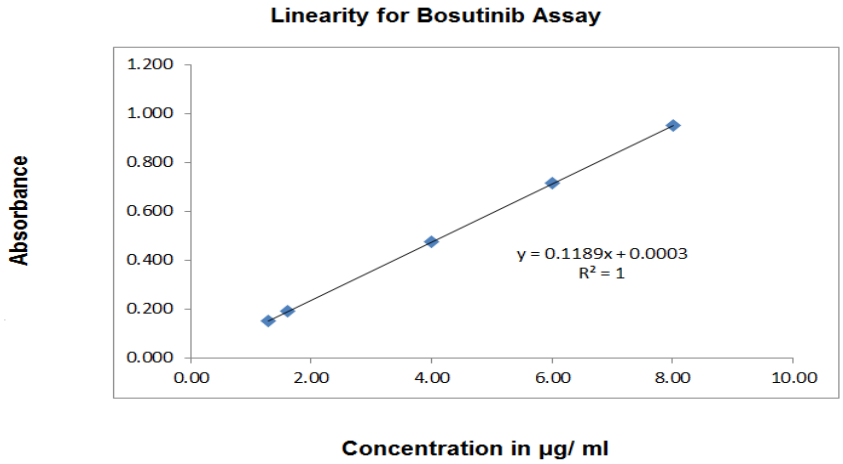

Fig. 19: Linearity graph of Bosutinib

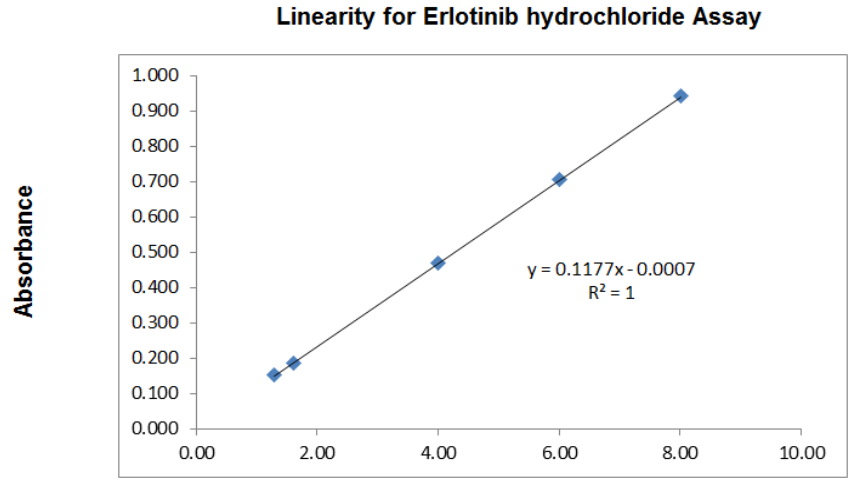

Fig. 20: Linearity graph of Erlotinib hydrochloride

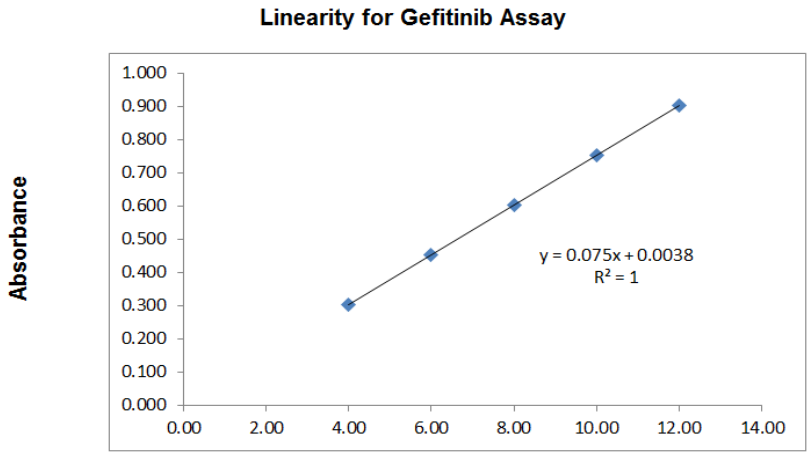

Fig. 21: Linearity graph of Gefitinib

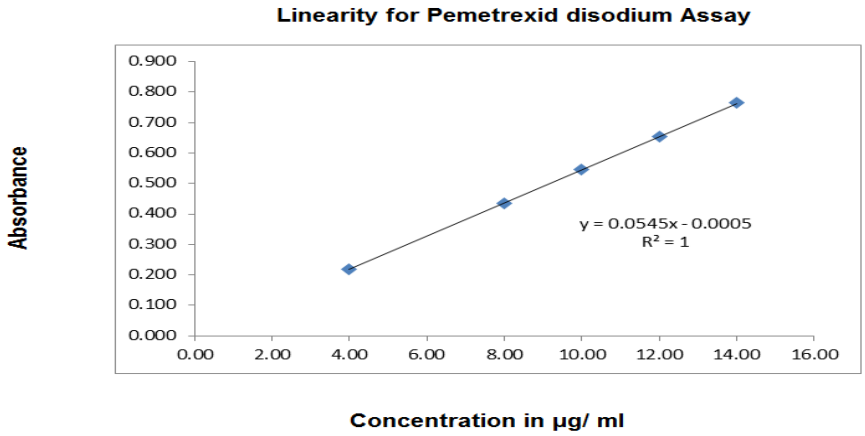

Fig. 22: Linearity graph of Pemetrexed Disodium

Table 3: Optical characteristics and Linearity data

Parameter	Axitinib	Bosutinib	Erlotinib hydrochloride	Gefitinib	Pemetrexed Disodium
Detection Wavelength (λ_{max})	336nm	269nm	247nm	250nm	225nm
Beer's Law limits (µg/ml)	4-12 (µg/ml)	1.28-8.01 (µg/ml)	1.28-8.00 (µg/ml)	4-12 (µg/ml)	4.00-14.01 (µg/ml)
Regression Statistics					
Slope	0.0871	0.1189	0.1177	0.0750	0.0545
Intercept	0.0065	0.0003	0.0007	0.0038	0.0005
Correlation coefficient	1.0000	1.0000	1.0000	1.0000	1.0000
Coefficient of determination (R^2)	1.0000	1.0000	1.0000	1.0000	1.0000
Intercept at 95% confidence interval (lower value–upper value)	0.01408- 0.00115	0.0044- 0.00492	0.0054- 0.00404	0.00233- 0.00527	0.00501- 0.00402
Slope at 95% confidence interval (lower value–upper value)	0.08622- 0.08801	0.11799- 0.11989	0.11678- 0.11870	0.07481- 0.07516	0.05408- 0.05497

Accuracy

The percentage recovery results for Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium were varied from 98.4%–100.4%, 98.5%–100.1%, 99.7%–100.4%, 99.7%–100.5% and 99.8%–100.5% respectively at three different concentration levels and the results were shown in Table 4 and Table 5. Based on the % recovery data, it was concluded that the developed method is capable for the estimation of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances and is adequate for routine analysis.

Table 4: Accuracy results of Axitinib, Gefitinib and Pemetrexed Disodium

%Accuracy Level for Axitinib and Gefitinib	% Recovery range for triplicate preparations	% Recovery range for triplicate preparations	%Accuracy Level for Pemetrexed Disodium	% Recovery range for triplicate preparations
	% Axitinib	% Gefitinib		% Pemetrexed Disodium
50%	98.4- 98.7	99.9- 100.5	40%	100.0- 100.5
100%	99.7- 100.0	99.9- 100.2	100%	99.8- 100.2
150%	100.2- 100.4	99.7- 100.0	140%	99.9- 100.0

Table 5: Accuracy results of Bosutinib and Erlotinib hydrochloride

%Accuracy Level for Bosutinib	% Recovery range for triplicate preparations	%Accuracy Level for Erlotinib hydrochloride	% Recovery range for triplicate preparations
	% Bosutinib		% Erlotinib hydrochloride
32%	98.5- 99.8	32%	99.7- 100.3
100%	99.4- 99.8	100%	100.1- 100.4
200%	99.6- 100.1	200%	100.0- 100.2

Robustness

The robustness of the proposed method was performed by preparing the standard solutions and test solutions of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium at 100% level and were analysed by a change in wavelength for absorbance readings. The wavelength selected was ± 2nm to the λ_{max} i.e. 334 and 338 nm for Axitinib, 267 and 271 nm for Bosutinib, 245 and 249 nm for Erlotinib hydrochloride, 248 and 252 nm for Gefitinib, 223 and 227 nm for Pemetrexed Disodium respectively for standard and sample solutions. In the robustness condition (wavelength variation of ± 2nm to the λ_{max}), the assay values of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium were found to be not less than 99%. %Assay results for the robustness parameter was shown in Table 6 and Table 7.

Table 6: Robustness results for Axitinib, Bosutinib and Pemetrexed Disodium

Determination	% Assay of Axitinib at 334nm	% Assay of Axitinib at 338nm	% Assay of Bosutinib at 267nm	% Assay of Bosutinib at 271nm	% Assay of Pemetrexed Disodium at 223nm	% Assay of Pemetrexed Disodium at 227nm
Determination-1	99.8	99.9	100.1	100.0	100.0	100.0
Determination-2	99.9	99.9	100.2	100.1	99.8	99.7
Determination-3	99.7	99.8	99.9	100.0	99.9	100.1
Average	99.8	99.9	100.1	100.0	99.9	99.9
SD	0.11	0.04	0.16	0.06	0.06	0.23
%RSD	0.11	0.04	0.16	0.06	0.06	0.23

Table 7: Robustness results for Erlotinib hydrochloride and Gefitinib

Determination	% Assay of Erlotinib hydrochloride at 245nm	% Assay of Erlotinib hydrochloride at 249nm	% Assay of Gefitinib at 248nm	% Assay of Gefitinib at 252nm
Determination-1	99.9	99.9	99.7	99.8
Determination-2	100.0	100.0	99.6	99.8
Determination-3	99.8	100.1	99.5	99.7
Average	99.9	100.0	99.6	99.8
SD	0.08	0.08	0.12	0.06
%RSD	0.08	0.08	0.12	0.06

Solution stability

The percent assay value difference was determined for solutions stored at room temperature and at refrigerated condition (2- 8°C) for different time intervals up to 24 hours. Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium absorbances were found to be stable up to 24 hours at room temperature and also at refrigerator condition. Solution stability results at room temperature and refrigerated condition were shown in Table 8.

Table 8: Solution stability results of standard and control sample at room temperature and at refrigerated condition.

Solution Stability	% Assay results				
Axitinib Solution Stability	**Initial**	**After 6 Hours**	**After 12Hours**	**After 24Hours**	**% Difference**
% Assay of standard solution at RT	99.8	99.9	99.6	99.5	0.3
% Assay of sample solution at RT	99.8	99.9	99.5	99.3	0.6
% Assay of standard solution at 2-8°C	99.8	99.8	99.9	99.8	0.1
% Assay of sample solution at 2-8°C	99.8	99.8	99.6	99.5	0.3
Bosutinib Solution Stability					
% Assay of standard solution at RT	99.8	99.6	99.4	99.2	0.6
% Assay of sample solution at RT	99.8	99.6	99.4	99.6	0.4
% Assay of standard solution at 2-8°C	99.8	100.0	99.8	99.6	0.4
% Assay of sample solution at 2-8°C	99.8	99.6	99.4	99.2	0.6
Erlotinib HCl Solution Stability					
% Assay of standard solution at RT	100.1	99.9	99.7	99.5	0.6
% Assay of sample solution at RT	99.9	99.7	99.4	99.2	0.7
% Assay of standard solution at 2-8°C	100.1	100.1	99.7	99.5	0.6
% Assay of sample solution at 2-8°C	99.9	99.7	99.7	99.7	0.2
Gefitinib Solution Stability					
% Assay of standard solution at RT	99.9	99.7	99.5	99.2	0.7
% Assay of sample solution at RT	100.0	99.8	99.8	99.5	0.5
% Assay of standard solution at 2-8°C	99.9	100.0	99.9	99.5	0.5
% Assay of sample solution at 2-8°C	100.0	99.6	99.5	99.3	0.7

Pemetrexed Disodium Solution Stability					
% Assay of standard solution at RT	99.9	99.7	99.5	99.3	0.6
% Assay of sample solution at RT	100.0	99.8	99.6	99.6	0.4
% Assay of standard solution at 2-8°C	99.9	99.9	99.7	99.3	0.6
% Assay of sample solution at 2-8°C	100.0	100.0	99.8	99.6	0.4

CONCLUSIONS

Simple, precise and economical UV-Visible spectrophotometric method has been developed for the quantitative estimation of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium in its active pharmaceutical ingredient form. Method is validated as per the ICH guidelines and also the developed method is robust with respect to wavelength variation of the original wavelength. The developed method can be used for the quantification of Axitinib, Bosutinib, Erlotinib hydrochloride, Gefitinib and Pemetrexed Disodium drug substances in routine analysis.

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REFERENCES

1. Available from http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202324lbl.pdf.
2. Available from https://en.wikipedia.org/wiki/Axitinib
3. Available from http://www.scbt.com/datasheet-217679-axitinib.html.
4. Available from https://en.wikipedia.org/wiki/Bosutinib
5. Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203341lbl.pdf.
6. Available from https://en.wikipedia.org/wiki/Erlotinib.
7. Available from http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021743s010lbl.pdf.
8. Available from https://en.wikipedia.org/wiki/Gefitinib.
9. Available from http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206995s000lbl.pdf.
10. Available from https://en.wikipedia.org/wiki/Pemetrexed.
11. Available from http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004114/WC500194694.pdf.
12. Chandra R, Sarada NC. Development and validation of stability indicating RP-HPLC method for the determination of Axitinib in Bulk and its Pharmaceutical Formulations. Der Pharmacia Lettre, 2016; 8(11): 97-106.
13. Lakshmi B, Saraswathi K, Reddy TV. RP-HPLC method development and validation for the analysis of Axitinib in pharmaceutical dosage forms. Int J Scie Inno and Discoveries, 2012; 2(1): 184-190.
14. Jadhav PB, Gajare GK. Development and validation of an RP-HPLC method for Bosutinib in bulk form. Int J Res in Pharm and Chemistry, 2016; 6(3): 599-603.
15. Kalyana CV, Gowri SD. Development and validation of RP-HPLC method for estimation of Erlotinib in bulk and its pharmaceutical formulation. Ras J Chemistry, 2011; 4(2): 393-399.
16. Faivre L, Gomo C, Mir O, Taieb F, Schoemann TA, Ropert S, Vidal M, Dusser D, Dauphin A, Goldwasser F, Blanchet B. A simple HPLC-UV method for the simultaneous quantification of gefitinib and erlotinib in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci, 2011; 879(23): 2345-50.
17. Saravanan G, Suryanarayana MV, Jadhav MJ, Ravikumar M, Someswararao N, Acharyulu PVR. A Stability-ndicating LC Assay Method for Pemetrexed Disodium. Chromatographia, 2007; 66(5): 431–34.
18. USP 37, NF 32. United States Pharmacopeial Convention, Rochville, Md, USA, 2014.
19. European Pharmacopoeia 8.7.
20. ICH, Q2(R1), harmonized tripartite guideline, validation of analytical procedures: text and methodology in proceedings of the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, 2005.
21. Ashok CV, Sailaja BBV. Method development and validation of assay and dissolution methods for the estimation of Daclatasvir in tablet dosage forms by reverse phase HPLC. European J of Pharma and Medical Research, 2016; 3(7): 356-64.
22. Ashok CV, Sailaja BBV, Praveen KA. Development and validation of a dissolution method for Frovatriptan tablets by reverse phase UPLC. International J of Pharm and Pharm Sciences, 2015; 7(4): 125–130.
23. Ashok CV, Sailaja BBV, Praveen KA. Stability indicating reverse phase high performance liquid chromatographic method for simultaneous estimation of Labetalol and its degradation products in tablet dosage forms. Asian J of Pharm and Clin Research, 2016; 9(2): 242–49.

24. United States Food and Drug Administration. Guidance for industry: Analytical procedures and methods validation: chemistry, manufacturing, and controls documentation. Draft Guidance, USFDA; 2001.
25. USP 37, NF 32. Validation of compendial methods, Chapter 1225. The United States Pharmacopeial Convention, Rockville, Md, USA; 2014.