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A review on analytical method development and validation of Erlotinib

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Abstract

In this review article determines the different analytical methods for the quantitative establishment of Erlotinib by using HPLC, HPLCMS, HPLC-UV, LC-MS/MS. Pharmaceutical analytical method development of Erlotinib requires valid analytical procedures for quantitative and qualitative analysis in Pharmaceuticals dosage formulations and human serum. This assessment explains that the superiority of the HPLC/LC-MS methods reviewed is based on the quantitative analysis of drugs in formulations, (API), biological fluids such as serum and plasma.

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Introduction

Erlotinib, sold under the brand name Tarceva among others, is a medication used to treat non-small cell lung cancer (NSCLC) and pancreatic cancer [1]. Specifically it is used for NSCLC with mutations in the epidermal growth factor receptor (EGFR) either an exon 19 deletion (del19) or exon 21 (L858R) substitution mutation — which has spread to other parts of the body [4]. It is taken by mouth.

Common side effects include rash, diarrhea, muscle pain, joint pain, and cough [2][3]. Serious side effects may include lung problems, kidney problems, liver failure, gastrointestinal perforation, stroke, and corneal ulceration [4]. Use in pregnancy may harm the baby [4]. It is a receptor tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR) [5].

Medical uses

Erlotinib in unresectable non-small cell lung cancer when added to chemotherapy improves overall survival by 19%, and improved progression-free survival (PFS) by 29%, when compared to chemotherapy alone [6][7]. The U.S. Food and Drug Administration (FDA) approved Erlotinib for the treatment of locally advanced or metastatic non-small cell lung cancer that has failed at least one prior chemotherapy regimen [8].

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. [9] [10] Overall survival, progression-free survival and one-year

survival are similar to standard second-line therapy (docetaxel or pemetrexed). Overall response rate is about 50% better than standard second-line chemotherapy [11]. 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 has been developed by Genzyme [12].

Pancreatic cancer

In November 2005, the FDA approved Erlotinib in combination with gemcitabine for treatment of locally advanced, unresectable, or metastatic pancreatic cancer [13].

Mechanism

Erlotinib is an epidermal growth factor receptor inhibitor (EGFR inhibitor). The drug follows Iressa (gefitinib), which was the first drug of this type. Erlotinib specifically targets the epidermal growth factor receptor (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. It binds in a reversible fashion to the adenosine triphosphate (ATP) binding site of the receptor [25]. For the signal to be transmitted, two EGFR molecules need to come together to form a homodimer. These then use the molecule of ATP to trans-phosphorylate each other on tyrosine residues, which generates phosphotyrosine residues, recruiting the phosphotyrosine-binding proteins to EGFR to assemble protein complexes that transduce signal cascades to the nucleus or activate other cellular biochemical processes. When Erlotinib binds to EGFR, formation of phosphotyrosine residues in EGFR is not possible and the signal cascades are not initiated.

Review on Erlotinib

Chahbouni reported that developed and validated liquid chromatography (LC)-mass spectrometry (MS)/MS method in human plasma for the tyrosine kinase inhibitors Erlotinib, gefitinib, and imatinib in human plasma. Pre-treatment of the samples was achieved by using liquid-liquid extraction and imatinib as internal standard. Separation was performed on a Waters Alliance 2795 LC system using an XBridge RP18 column. The mass spectrometer Micromass was equipped with an electro spray ionization probe, operating in the positive mode. The calibration curves in plasma were linear for erlotinib, gefitinib, and imatinib over the concentration range of 5 to 3,000; 5 to 3,000, and 5 to 5,000 ng/mL, respectively. The intraday and interday accuracy ranged from 90% to 110% and the intraday and interday precision of the method was within 5%.

R. Honeywell developed a simple and selective method for the determination of various tyrosine kinase inhibitors by liquid chromatography tandem mass spectrometry. Utilizing a simple protein precipitation with acetonitrile a 20 μL sample volume of biological matrixes can be extracted at 4 °C with minimal effort. After centrifugation the sample extract is introduced directly onto the LC–MS/MS system without further clean-up and assayed across a linear range of 1–4000 ng/ml. Chromatography was performed using a Dionex Ultimate 3000 with a Phenomenex prodigy ODS3 (2.0 mm × 100 mm, 3 μm) column and eluted at 200 μL/min with a tertiary mobile phase consisting of 20 mM ammonium acetate: acetonitrile: methanol (2.5:6.7:8.3%). Injection volume varied from 0.1 μL to 1 μL depending on the concentration of the drug. Samples were observed to be stable for a maximum of 48 h after extraction when kept at 4 °C. Detection was performed using a turbo-spray ionization source and mass spectrometric positive multi-reaction-monitoring-mode (+MRM) for Gefitinib (447.1 m/z; 127.9 m/z), Erlotinib (393.9 m/z; 278.2 m/z), Sunitinib (399.1 m/z; 283.1 m/z) and Sorafenib (465.0 m/z; 251.9 m/z) at an ion voltage of +3500 V. The accuracy, precision and limit-of-quantification (LOQ) from cell culture medium were as follows: Gefitinib: 100.2 ± 3.8%, 11.2 nM; Erlotinib101.6 ± 3.7%, 12.7 nM; Sunitinib: 100.8 ± 4.3%, 12.6 nM; Sorafenib: 93.9 ± 3.0%, 10.8 nM, respectively. This was reproducible for plasma, whole blood, and serum. The method was observed to be linear between the LOQ and 4000 ng/ml for each analyte.

V.Rajeshdeveloped a simple, specific and precise high performance thin layer chromatographic method for estimation of Erlotinib hydrochloride as bulk drug. The chromatographic development was carried out on Precoated silica gel 60 F254 aluminium plates using mixture of Methanol: Ammonia (8:0.2 v/v) as mobile phase and Densitometric evaluation of band was carried out at 250 nm using Camag TLC Scanner-3 with win CAT 1.4.3 version software. The RF value of drug was found to be 0.52 ± 0.01. The method was

validated with respect to linearity, accuracy, precision and robustness. The calibration curve was found to be linear over a range of 200- 1200 ng/ band. The % assay (Mean ± S.D.) was found to be 101.3 ± 1.02. The proposed HPTLC method was found to provide a faster and cost effective quantitative control for routine analysis of Erlotinib hydrochloride as bulk drug.

Faivre L, Gomo: developed a simple HPLC-UV method for the simultaneous quantification of gefitinib and erlotinib in human plasma. Gefitinib and erlotinib are two oral tyrosine kinase inhibitors (TKI). Following liquid-liquid extraction, gefitinib, erlotinib and sorafenib (internal standard), were separated with gradient elution using C8 column and mobile phase of acetonitrile/20mM ammonium acetate pH 4.5. Samples were eluted at a flow rate of 0.4 ml/min throughout the 15-min run. Dual UV wavelength mode was used, with gefitinib and erlotinib monitored at 331 nm, and sorafenib at 249 nm. The calibration was linear in the range 20-1000 ng/ml and 80- 4000 ng/ml for gefitinib and erlotinib, respectively. Inter- and intra-day precision were less than 7.2% and 7.6% for gefitinib and erlotinib, respectively.

G.Usha Rani: developed and validated extractive colorimetric method for estimation of erlotinib in bulk and tablet dosage form. Two simple, rapid sensitive, precise and economic spectrophotometric methods for the estimation of erlotinib. The solution of the drug formed colored ion-pair complexes with Bromocresol Green (BCG) and Methyl Orange (MO) in phosphate buffer pH 2.5, and extracted in chloroform. The complex of etoricoxib with BCG and MO showed λ max at 418.5nm and 424.4nm respectively. The complex was stable up to 22 hrs and obeyed Beer's law over the concentration ranges of 10-1000 ug/ml. Correlation coefficient was found to be 0.9985.

M.Padmalatha: developed a spectrophotometric method for the determination of erlotinib in pure and pharmaceutical dosage form. The developed methods were based on reaction of erlotinib with Phenol red, BromoCresolGreen and Erichrome black T. They are quantified spectrophotometrically at their absorption maximum at 418nm (MethodA), 424nm (MethodB) and 333nm (MethodC). Beer's law was obeyed in the concentration range of 40-80ug/ml, 20-40ug/ml and 10-50ug/ml for the three method respectively. The colors were found to be stable for more than 4 hrs.

V.Kalyana Chakravarthy: development and validated of RP HPLC method for estimation of erlotinib in bulk and its pharmaceutical formulation. A RP-LC method used a kromasil 100-5, C18(150mm×4. 6mmi.d; particle size 5um) and potassium dihydrogen phosphate buffer pH 2.4: acetonitrile:methanol (65:21:14) as eluent at flow rate 1.5ml/min with UV detection at 250 nm. The erlotinib content was linear over a range of 75.02 to 225.06 ug/ml. The method precision for the determination of assay was below 2.0 %RSD. The percentage recoveries of active pharmaceutical ingredients from dosage forms ranged from 100.5 to 101.1.

G. Vidya Sagar: developed and validated a simple, accurate and cost efficient spectrophotometric method, for the estimation of erlotinib in tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wave length (λmax) was found to be 247 nm. The percentage recovery of erlotinib was in the range of 99.7±0.12. Beer's law was obeyed in the concentration range of 2- 10ug/ml. Calibration curves showed a linear relationship between the absorbance and concentration.

M.Padmalatha: developed and validated High Performance Liquid Chromatographic Method for the determination of Erlotinib. They used 250x4.6mm, 5µ particle, IntersilODS-3V C18column with 0.03M potassium dihydrogen orthophosphate in water pH 3.2, orthophosphoric acid acetonitrile (55:45), as mobile phase at a flow rate of 0.8 ml/min. PDA detection was performed at 246. 0nm.Injection volume was20µl.HPLC grade water, Acetonitrile(50:50v/v) was used as diluents. The method was validated for accuracy, precision, linearity, specificity and sensitivity. Total run time was 20min, erlotinib eluted with retention time of 4. 75min.Calibration plots were linear over the concentration range 5-40µg/ml. Intra and inter day relative standard deviation for erlotinib was less than 3.3 and 4.1% respectively.

Luca Signor: reported analysis of erlotinib and its metabolites in rat tissue sections by MALDI quadrupole time-of- flight mass spectrometry. The analysis was carried out on rat tissue sections from liver, spleen and muscle. Following oral

administration at a dose of 5mg/kg, Samples were analyzed by matrix assisted laser desorption ionization (MALDI) with mass spectrometry (MS) using a orthogonal quadrupole time of flight instrument. The presence of the parent compound and of its o-demethylated metabolites was confirmed in all tissues types and their absolute amounts calculated. In liver the intact drug was found to be 3.76ng/mg tissue, while in spleen and muscle 6-30 folds' lower values. These results were compared with drug quantitation obtained by whole-body autoradiography, which was found to be similar.

Lutz Gotze: development and clinical application of a LC/MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma. Developed and validated a specific, simple and rapid quantification method for various TKI's in human plasma. A simultaneous test for six TKI's (erlotinib, imatinib,lapatinib, nilotinib, sorafenib, Sunitinib) was developed using liquid chromatography tandem mass spectrometry in a multiple reaction monitoring mode. After protein precipitation the specimens were applied to the HPLC system and separated using a gradient of acetonitrile containing 1% formic acid with 10mM ammonium formate on an analytic RP C18 column. The calibration range was 10- 1000ng/ml for Sunitinib and 50-5000ng/ml for the other TKI's with coefficient of determination ≤15% and the chromatographic run time was 12 min. Plasma specimens were stable for measurement for atleast 1 week at 4°c.

S.S Pujeri: developed and validated stability – indicating chromatographic method for the assay of erlotinib active pharmaceutical ingredient in the presence of its degradation products on a C18 column using a mobile phase of 0.01M ammoniumformate-acetonitrile-containing formic acid with a flow rate of 1.0ml/min. Selectivity was validated by subjecting the stock solution of erlotinib to acidic, basic, photolysis, oxidative and thermal degradation. The linearity range and values for limit of detection (LOD) and quantification (LOQ) were found to be 1-198, 0.33, and 1.1ug/ml, respectively. The analysis of the tablet containing erlotinib was quite precise (relative standard deviation)

ErrinR.Lepper: developed and validated a high-performance Liquid Chromatographic (HPLC) assay with U.V detection for the quantitative determination of erlotinib in human plasma. Quantitative extraction was achieved by a single-solvent extraction involving a mixture of acetonitrile and n-butyl chloride(1:4v/v). Erlotinib and the internal standard hydrochloride salt (OSI-597) were separated on a column packed with NOVA-PAK C18 material and a mobile phase composed of acetonitrile and water, pH 2.0 (60:40, v/v).The column effluent was monitored with dual U.V detection at wavelengths of 348nm erlotinib and 383nm erlotinib hydrochloride. The calibration graph was linear in the range of 100- 4500ng/ml, with values for accuracy and precision ranging from 87.9 to 96.2% and 2.13 to 5.10% respectively, for three different sets of quality control samples.

Rasoulzadeh: studied the mutual interaction of anticancer drug erlotinib hydrochloride with bovine serum albumin (BSA) using fluorescence and U.V /VIS spectroscopy. The BSA solution(0.1Mm) was prepared daily in tris buffer (0.05mol1,ph=7.4) and treated at final concentration of 1.67x10-5M with different amount of erlotinib hydrochloride to obtain final concentration of 0,0.2,0.4,0.8,1,2,4,6,8,20 and 42μm respectively. The mixture was allowed to stand for 5 min and the fluorescence quenching spectra were recorded at 298,303, 308 and 313k. It was found that erlotinib hydrochloride caused the fluorescence quenching of BSA by the formation of a BSA –ERLOTINIB HYDROCHLORIDE complex. The mechanism of the complex formation was then analysed by determination of the number of binding sites the apparent binding constant Ka, and calculation of the corresponding thermodynamic parameters. Such as the free energy (ΔG), enthalpy (ΔH) and entropy changes (ΔS) at different temperatures. Results showed that binding of erlotinib hydrochloride to BSA was spontaneous and the hydrophobic forces played a major role in the complex formation. The distance r between donor (BSA) and the acceptor (ERLOTINIB HYDROCHLORIDE) was found to be less than 8nm. Non-radioactive energy transferring and static quenching between these two molecules. The presence of single binding site on BSA and Ka values for the association of BSA

with ERLOTINIB HYDROCHLORIDE increased by the increase in temperature.

Jiongwei Pan: developed a novel bioanalytical method and validated for the quantitative determination of erlotinib in human plasma by using the supported liquid extraction (SLE), sample cleanup coupled with hydrophilic interaction liquid chromatography and tandem mass spectrometric detection (HILIC-MS/MS). The SLE extract could be directly injected into the HILIC-MS/MS system for analysis without the solvent evaporation and reconstitution steps. Erlotinib was used as the internal standard. The SLE extraction recovery was 101.3%. The validated linear curve range was 2 to 2,000 ng/mL based on a sample volume of 0.100-mL, with a linear correlation coefficient of > 0.999. The validation results demonstrated that the present method gave a satisfactory precision and accuracy: intra-day CV < 5.9% (< 3.2% (((<1.5% for LLOQ) with n = 18 and the accuracy of 100.0– 103.2%. A dilution factor of 10 with blank plasma was validated for partial volume analysis. The stability tests indicated that the erlotinib in human plasma is stable for three freeze-thaw cycles (100.0–104.5% of the nominal values), or 24-h ambient storage (100.0–\104.8% of the nominal values), or 227-day frozen storage at both -20 ºC (91.5–94.5% of the nominal values) and -70 ºC (93.3–93.8% of the nominal values). The results also showed no significant matrix effect (<6.3%) even with direct injection of organic extract into the LC-MS/MS system.

Fouad Chiadmi: developed and validated an iso cratichigh-performan celiquid chromatographic method for the determination of erlotinib in human plasma with detection at348nm. Quinine was used as internal standard. Areversed-phasesymmetry C18 column (250 mm x 4.6 mm, 5 μm), was equilibrated with a mobilephase composed of potassium dihydrogen phosphate 0.05M and acetonitrile (60:40,v/v) with a final pH of 4.8 and having a flow rate of 1 mL/minute. The elution time for erlotinib and internal standard wasapproximately7.4 and 2.6 minutes, respectively. Calibration curves of erlotinib in human plasma were linear in theconcentration range of 50-1,000 ng/mL. Limits of detection and quantification inplasma were 6.3 and 21 ng/mL, respectively. Intra- and inter-day relative standard deviation for erlotinibin plasma was less than 3.3 and4.1%, respectively.

Hanqing Li et.al: developed a new synthetic and differential anti-proliferativeactivity of two active isomeric metabolites of Erlotinib were investigated. This synthetic process had demonstrated to avoid the unstable 4-chloro-quinazoline intermediates and long procedures. New intermediates and final compounds were identified by^{1} HNMR, ^{13} CNMR and their purities were determined by HPLC. Invitro proliferative assay indicates that these two metabolites possessed anti-proliferativeactivity against some conventional tumor cell lines and EGFR tyrosine kinase over-expression tumor cell lines as compared to Erlotinib control and their antitumor activity in cellular level was reported.

Han-Qing: developed and validated a new HPLC-UV method for thequantitative determination of epidermal growth factorreceptor inhibitor erlotinib inthe plasma of tumor bearing BALB/c nude mice. Erlotinib and its internal standard1-(3-((6,7-bis (2-methoxyethoxy) quinazolin-4-yl) amino) phenyl) ethanone wereextracted from mice plasma samples using liquid-liquid extraction with a mixedsolvent of methyl t-butyl ether and ethyl acetate (9:1, v/v). Luna C18 column (4.6mm×250 mm, 5 μm) with acetonitrile: 5 mM potassium phosphate buffer pH = 5.2(41:59, v/v) as the mobile phase. UV detector was set at the wavelength of 345 nm, and the flow rate was1.0mL/min. The calibration curve was linear over the range of 20–10 000 ng/mL with acceptable intra- and inter-day precision and accuracy. Theintra-day and inter-day precisions were within the range of 1.69%–5.66%, and the accuracies of intra- and inter-day assays were within the range of 105%–113%. The mean recoveries were 85.2% and 96.1% for erlotinib and internal standard, respectively.

Rajesh: developed a simple and sensitive spectrofluorometric method for the estimation of erlotinib hydrochloride in pure and pharmaceutical dosage forms. Erlotinib

hydrochloride exhibits maximum fluorescence intensity in methanol and the Beer's law was obeyed in the range of1-5µg/m Latan excitation wave length (λex) of 295 nm and an emission wave length (λem) of 339 nm. Stability studies with respectto time and temperature were also carried out. The results obtained were in good agreement with the labelled amounts of the marketed formulations. This method has been statistically evaluated and found to be accurate and precise**.**

Conclusion

A sensitive and accurate RP-HPLC methods, stability-indicating HPLC, HPLC-PDA, HPLC-UV, stability indicating HPTLC and HPLC-MS, with solid phase extraction methods was developed for the estimation of erlotinib, in pharmaceutical dosage forms, human plasma, the above methods was evaluated for Specificity, Linearity, Accuracy, Precision, Ruggedness and Robustness as per ICH&FDA guidelines.

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