Research article

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An eco-friendly direct spectrofluorimetric method for the determination of irreversible tyrosine kinase inhibitors, neratinib and pelitinib: application to stability studies

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ABSTRACT: A new rapid and simple stability-indicating spectrofluorimetric method has been developed for the determination of two irreversible tyrosine kinase inhibitors (TKIs), neratinib (NER) and pelitinib (PEL). The method is based upon measurement of the native fluorescence intensity of both drugs at λ_{ex} 270 nm in aqueous borate buffer solutions (pH 10.5). The fluorescence intensity recorded at 545 nm (NER) and 465 nm (PEL) were rectilinear over the concentration range of 0.1–10 μg/mL for both drugs with a high correlation coefficient ($r > 0.999$). The proposed method provided low limits of detection and of quantitation of 0.07, 0.11 μg/mL (NER) and 0.02, 0.05 μg/mL (PEL), respectively. The method was successfully applied for the determination of NER and PEL in bulk powder. The proposed methods were fully validated as per the International Conference on Harmonisation (ICH) guidelines. The application of the method was extended to stability studies of both NER and PEL under different forced-degradation conditions (acidic-induced, base-induced, oxidative, wet heat, and photolytic degradation). Moreover, the kinetics of the base-induced and oxidative degradation of both drugs was investigated and the pseudo-first-order rate constants and half-lives were estimated at different temperatures. Also, an Arrhenius plot was applied to predict the stability behaviour of the two drugs at room temperature. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: spectrofluorimetry; neratinib; pelitinib; stability studies; degradation kinetics

Introduction

Lung cancer is the main cause of cancer mortality worldwide, of which non-small cell lung cancer (NSCLC) is the most predominant of all the lung cancer cases. Generally, lung cancer is associated with poor prognosis (1,2). The human genome encodes for hundreds of tyrosine kinases that are responsible for the regulation of different physiological mechanisms, including cell proliferation, differentiation, migration, and metabolism. Dysfunction in kinase activity results in a disruption of the normal control of cellular phosphorylation signalling pathways with numerous abnormalities leading to cancer (3,4). This situation has encouraged the development of small molecule tyrosine kinase inhibitors (TKIs) as targeted therapy against tyrosine kinases, particularly epidermal growth factor receptors (EGFR). Most of these compounds target the ATP binding site across the human protein kinases. The first emerged class of TKIs (e.g. gefitinib, erlotinib) was designed to inhibit protein catalytic activity in a reversible manner through binding to the kinase domain of the target by weak interactions (hydrogen bonds, van der Waals forces and hydrophobic interactions). In the past decade, much progress has been made in the development of a new class of potent and selective TKIs that irreversibly inhibit their target protein by the formation of covalent bonds (1). These covalent irreversible TKIs constitute the second-generation TKIs. Several theoretical advantages of the second-generation irreversible EGFR TKIs over the first-generation reversible EGFR TKIs are that some of these have prolonged pharmacological effect, long duration of action, and a higher affinity and selectivity for the EGFR kinase domain, and may allow a more complete blockade of the EGFR signalling pathway (1,5). Furthermore, covalent bond formation gives irreversible inhibitors the potential to overcome resistance, compared with the reversible inhibitors. The number of irreversible TKIs entering clinical trials studies is steadily increasing. Examples of these are neratinib (NER) and pelitinib (PEL) (Fig. 1) (1). NER has demonstrated antitumor activity and an acceptable safety profile in patients with breast cancer and other solid tumours (6–14), uterine serous carcinoma, and NSCLC (13). PEL is currently being tested in phase II clinical trials for its efficacy on NSCLC (13,15) and metastatic colorectal cancer (16).

A review of the literature revealed the absence of reports dealing with the analysis of NER/PEL in pharmaceutical matrices and that only few liquid chromatography tandem mass spectrometry (LC–MS/MS) methods have been reported for the analysis of NER (10–12,14) or PEL (15,16) in biological samples. As the effective

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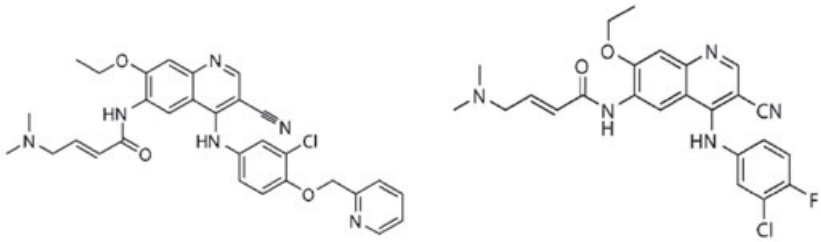
Abbreviations: EGFR, epidermal growth factor receptor; HPLC, high pressure liquid chromatography; ICH, International Conference on Harmonisation; LOD, limit of detection; LOQ, limit of quantitation; NSCLC, non-small cell lung cancer; PCT, Patent Cooperation Treaty; RSD, relative standard deviation; TKI, tyrosine kinase inhibitors; UV, ultraviolet.



NER PEL

Figure 1. Chemical structures of the studied compounds.

and safe therapy is dependent mainly on the homogeneity of bulk powder and uniformity of dosage units, accurate analytical methods for the analysis of the selected TKIs in pharmaceutical matrices are required. The designed work aims at development and validation of analytical methods for the determination of NER/PEL that could be easily employed in routine quality control of bulk powder and pharmaceuticals. Fluorescence spectroscopy was employed in this respect.

Fluorescence spectroscopy and time-resolved fluorescence have been widely applied as valuable research tools in modern analysis. Fluorescence measurement is now a widely used methodology that is applied extensively in biotechnology, forensics, and genetic analysis. Recently, developments in instrumentation and sample handling techniques, along with its high sensitivity and selectivity, have contributed to the wide application of fluorimetry in different aspects of drug analysis. Moreover, fluorimetric analysis has many advantages over other analytical techniques, and is generally more sensitive than classical ultraviolet (UV) light absorption detection, less expensive than LC–MS/MS detection, and simpler, more economical and time saving than high pressure liquid chromatography (HPLC) techniques (17–19). This technique has been applied previously in the analysis of some TKIs e.g. erlotinib (20,21) and gefitinib (22).

At this time, many efforts have been made to provide a direct orientation for greening the analytical procedures. The desire to replace hazardous substances with less polluting ones has increased. In this respect, certain principles have been set that focus on prevention, rather than remediation, of pollution effects of chemicals by providing general guidelines for improving the synthesis methods for the sake of reducing chemical toxicity of involved compounds (23,24). Moreover, these guidelines also focus on improving analytical methodologies to allow real-time and in-process monitoring and control prior to the formation of hazardous substances. In general, analytical procedures should focus on the replacement of toxic reagents and avoiding waste generation. Thus, direct measurements of untreated samples are considered the greenest methodologies without the use of toxic reagents and solvents (25).

To our knowledge, neither NER nor PEL in their bulk powder or pharmaceutical matrices has been determined previously. This encouraged us to study, for the first time, the native fluorescence of both NER and PEL in an attempt to develop a simple and sensitive spectrofluorometric method for their determination, either in bulk powder or in any future formulated preparations. The utility of the method was also extended to studying the stability of both drugs under different stress conditions, thus establishing the first stability-indicating study of both NER and PEL. Degradation kinetics was also conducted under base-induced and oxidative degradation. As this work depends on simply direct measurement of the fluorescence intensity of the analytes' solutions in aqueous borate buffer, without the need of any previous derivatization or the implication of hazardous solvent, this eco-friendly method serves as a typical example of green analytical procedures.

Experimental

Instrumentation

Fluorescence measurements were made on a SpectraMax (SpectraMax M5, Molecular devices, California, USA). Data acquisition was performed using SoftMax Pro v. 5.4® software (Molecular Devices, California, USA). All measurements were made using the following instrumental parameters: medium sensitivity, 5 nm step, and normal scan speed. pH measurements were carried out using a microprocessor laboratory pH meter (Mettler-Toledo International Inc., Zürich, Switzerland).

Materials and reagents

NER and PEL standards were purchased from Sigma-Aldrich (Saint-Louis, MO, USA). All solvents and reagents used were of analytical grade. Acetonitrile, methanol, hydrochloric acid 37% (PanReac AppliChem, Barcelona, Spain), borax and sodium dihydrogen orthophosphate anhydrous (Winlab Ltd., Leicestershire, UK), orthophosphoric acid 85% (BDH Laboratory Supplies Ltd., Poole Dorset, England), dimethylsulfoxide (DMSO) and dimethyl formamide (DMF) (Loba Chemie Pvt. Ltd., Mumbai, India), and absolute ethanol (Sigma-Aldrich Co., St. Louis, MO, USA) were used. Also, sodium hydroxide and boric acid (Central Drug House (P) Ltd., New Delhi, India), acetone (Fisher Scientific UK Ltd., Leicestershire, UK), and hydrogen peroxide (Merck KGaA, Darmstadt, Germany) were used. Analytical grade surfactants were also involved in the study; namely sodium dodecylsulfate (SDS) (Avonchem Ltd., Cheshire, UK), β-cyclodextrin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and Tween-80 (BDH Laboratory Supplies Ltd., Poole Dorset, UK). Glass double-distilled water was used throughout the study.

Standard solutions and calibration graphs

Stock solutions of 1000 μg/mL of NER and PEL were prepared in methanol. Intermediate stock solutions of 100 μg/mL of both drugs were prepared in borate buffer (pH 10.5). Accurate volumes of these intermediate stock solutions were diluted in the same buffer to prepare standard solutions of concentrations 0.1–10 μg/mL of each of NER and PEL.

The emission spectra of the standard solutions of both drugs were recorded in the range 250–700 nm following excitation at

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270 nm and corrected for the blank signal. Relative fluorescence intensities were recorded at 545 and 465 nm for the determination of NER and PEL, respectively. Calibration graphs were plotted by relating the emission intensities recorded at the selected wavelengths against the corresponding concentration for both compounds. Alternatively the corresponding regression equations were derived.

Procedure for tablets

As commercial tablets of neither NER nor PEL have been marketed to date, laboratory-prepared tablets were used instead. They were prepared based on reported patents dealing with the preparation of NER (26) and PEL (27) tablets. Accordingly, laboratory-prepared tablets were prepared by mixing each drug with different excipients that could be encountered in actual tablets formulation (e.g. lactose, methyl cellulose, iron oxide etc.). An accurately weighed quantity of the prepared powder mixtures equivalent to 1 mg of NER or PEL was transferred into 10 mL volumetric flasks. Six millilitres of methanol were added to each flask and they were sonicated in an ultrasonic bath for 30 min, then diluted to volume with the same solvent and filtered. Portions of 100 μL of the previously prepared solutions were diluted with borate buffer pH 10.5 to a 10 mL volume to prepare simulated tablet solutions labelled to contain 1.0 μg/mL of each of NER and PEL. The emission spectra of these solutions were recorded in the range 250–700 nm following excitation at 270 nm and corrected for the blank signal. Relative fluorescence intensities were recorded at the selected emission wavelengths, as shown under construction of the calibration graphs. The nominal contents were calculated either from the previously constructed calibration graphs or using the corresponding regression equation for each of NER and PEL.

Application to stability studies

To test for the stability-indicating property of the proposed fluorimetric method, NER and PEL were subjected to forced-degradation studies under different stress conditions.

Acid-induced degradation

Volumes of 1000 μL of either NER or PEL stock solutions (1000 μg/mL) were separately mixed with 4 mL of 2 M hydrochloric acid solution then heated in a boiling water bath for 60 min.

Base-induced degradation

Separate volumes of 1000 μL of either NER or PEL stock solutions (1000 μg/mL) were mixed with 4 mL of 0.2 M sodium hydroxide solution. The reaction mixtures were then placed in a boiling water bath for 60 min.

For both acid- and base-induced degradation, the resultant solutions were left to cool at room temperature and then neutralized with pre-determined amounts of 2 M sodium hydroxide or 0.2 M hydrochloric acid for acid- and base-induced degradation, respectively. The neutralized solutions were then diluted to 10 mL with distilled water. Then portions of 500 μL were separately diluted to final volumes of 5 mL with borate buffer pH 10.5 to get concentrations equivalent to 10.0 μg/mL of each of NER and PEL.

Oxidative degradation

Volumes of 1000 μL of either NER or PEL stock solutions (1000 μg/mL) were separately mixed with 1 mL of 0.5% hydrogen peroxide solution. These solutions were then placed in a thermostated water bath at 90° C for 60 min. These solutions were then made up to 10 mL with distilled water. Final dilutions with borate buffer pH 10.5 to get concentrations equivalent to 10.0 μg/mL of each of NER and PEL were made exactly as under acid- and base-induced degradation.

Photochemical degradation

The photochemical stability of both NER and PEL was tested by exposing standard solutions (10 μg/mL) of each drug to direct daylight for 24 h, UV radiation at 254 and 366 nm for 2 h to test for daylight, short UV, and long UV stability, respectively.

Wet heat-induced degradation

Separate volumes of 1000 μL of either NER or PEL intermediate stock solutions (100 μg/mL) were separately heated in a boiling water bath for 60 min. Stressed samples were diluted to a 10-mL volume with borate buffer pH 10.5.

Degradation kinetics

Base-induced degradation. Into a series of glass tubes, separate aliquots of 1000 μL of either NER or PEL stock solutions (1000 μg/mL) were separately mixed with 4-mL volumes of 0.2 M sodium hydroxide solutions. The tubes were placed in a thermostated water bath at different temperatures (70, 80, 90 or 100 °C). At different time intervals (0, 10, 20, 30, 40, 50 or 60 min) specified tubes were withdrawn, cooled at room temperature, then neutralized with pre-determined amounts of 0.2 M hydrochloric acid solution.

Oxidative degradation. Into a series of glass tubes, separate aliquots of 1000 μL of either NER or PEL stock solutions (1000 μg/mL) were separately mixed with 0.5% hydrogen peroxide solution. The tubes were then heated in a thermostated water bath at different temperatures (60, 70, 80 or 90 °C) for different time intervals (0, 10, 20, 30, 40, 50 or 60 min).

In both base-induced and oxidative degradation, stressed samples were diluted to 10 mL volume with water then appropriate dilutions were made with borate buffer solution (pH 10.5) to get concentrations equivalent to 10 μg/mL of both drugs. The emission spectra of diluted samples were measured at the excitation wavelength of 270 nm. Using the previously constructed calibration graphs or regression equations, the relative fluorescence intensities recorded at 545 and 465 nm were used for the determination of the concentration of remaining NER and PEL, respectively. Data were further processed and degradation kinetics was investigated.

Results and discussion

Spectral characteristics

NER and PEL borate buffer solutions (pH 10.5) were found to exhibit native fluorescence at 545 and 465 nm for NER and PEL, respectively after excitation at 270 nm (Fig. 2).

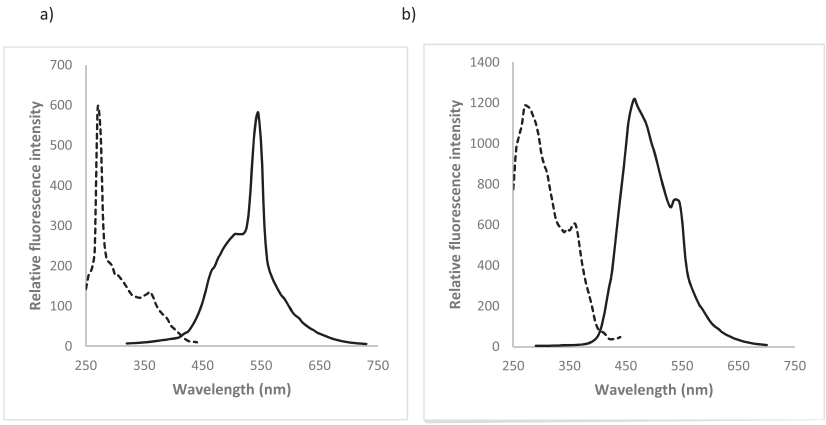

Figure 2. Excitation and emission fluorescence spectra of (a) neratinib (NER; 10 μg/mL); and (b) pelitinib (PEL: 10 μg/mL) in borate buffer pH 10.5. Dotted spectra stand for excitation while solid spectra stand for emission.

Factors affecting fluorescence intensity

Different factors affecting the fluorescence characteristics of both drugs were carefully studied. They include: type of diluting solvents, the effect of pH, and the inclusion of different surfactants.

Effect of diluting solvent. Different diluting solvents were tested for their effect on the fluorescence characteristics of both NER and PEL. They include: acetonitrile, methanol, ethanol, water, DMSO, and DMF. Solvents not only affect the fluorescence intensity, but also affect the position of the excitation and emission maxima of both drugs. It is clear from Fig. 3(a, a′) that water alone produces the maximum fluorescence intensity for NER compared with DMF for PEL.

Effect of pH. In order to investigate the influence of pH on the fluorescence intensity of both NER and PEL, separate solutions of both drugs were diluted with phosphate buffer (pH 3.0–7.5) and borate buffer (pH 9.5–11.0). It was clear from Fig. 3(b, b′) that

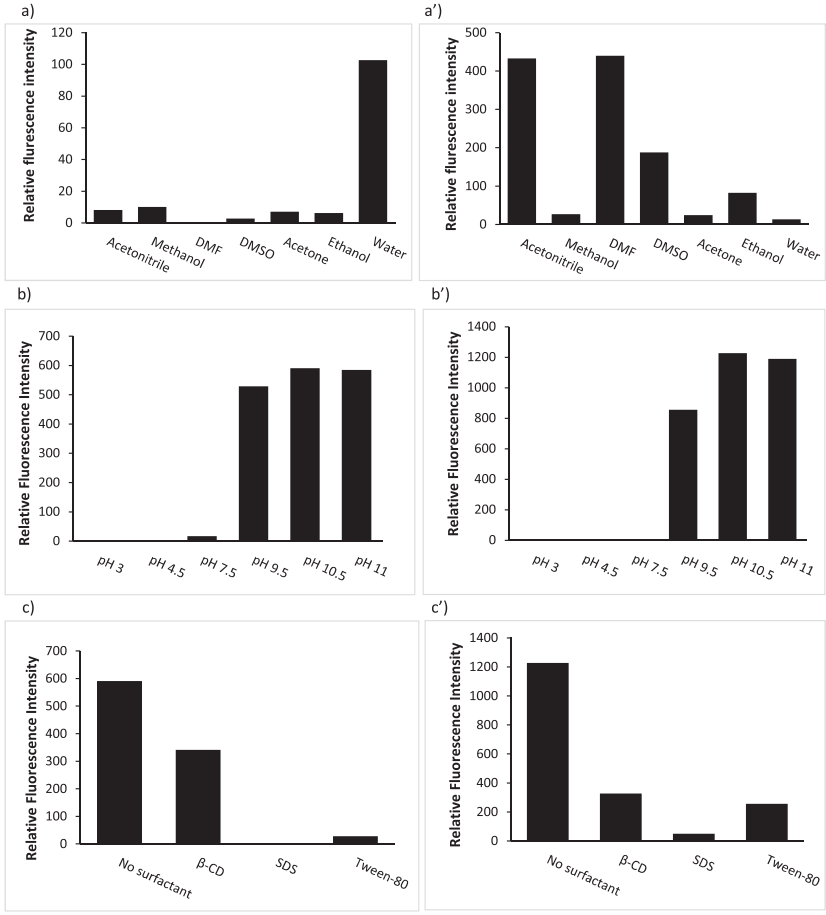

Figure 3. Illustration of factors affecting the relative fluorescence intensity of (a–c) neratinib (NER) 10 μg/mL; and (a′–c′) pelitinib (PEL) 10 μg/mL; as follows: (a, a′) effect of diluting solvents; (b, b′) effect of pH; and (c, c′) effect of different surfactants.

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borate buffer pH 10.5 provided maximum fluorescence intensity for both drugs. As both NER and PEL are basic compounds, increasing the pH resulted in higher quantum yield for the unprotonated bases, compared with the protonated form at low pH.

Effect of surfactants. Moreover, the effect of different surfactants on the relative fluorescence intensity of NER and PEL were tested. Aqueous solutions of 2% SDS, 2% β-cyclodextrin, and 2% Tween-80 were involved in the study. Thus, NER and PEL standards diluted with 2% aqueous surfactant solution:borate buffer pH 10.5 (20:80, v/v) were evaluated. It was observed that the use of borate buffer pH 10.5 alone with no surfactant produced the maximum fluorescence intensity of both dugs (Fig. 3c, c′). Therefore, no surfactant was included in this study.

As final measurements were performed using simply borate buffer pH 10.5 as a diluting solvent without the need of any organic modifiers, this method is considered eco-friendly.

Table 1. Regression and statistical parameters for the determination of NER and PEL by the proposed spectrofluorimetric method

Parameter	NER	PEL
Linearity range (μg/mL)	0.1–10	0.1–10
LOQ (μg/mL)	0.11	0.05
LOD (μg/mL)	0.07	0.02
Intercept (a)	4.3342	−9.6883
Slope (b)	57.715	107.319
Correlation coefficient (r)	0.9997	0.9992
S_a	1.1027	7.3301
S_b	0.2346	1.5597
$S_{y/x}$	1.9870	13.2080
F	13950.60	4733.97
Significance F	3.0814×10^{-8}	2.6735×10^{-7}

LOQ: limit of quantitation. LOD: limit of detection. S_a: standard deviation of intercept. S_b: standard deviation of slope. $S_{y/x}$: standard deviation of residuals. F: variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals).

Method validation

The proposed method was fully validated as per the ICH guidelines (28). Different validation aspects were evaluated including, linearity, limits of detection and of quantitation, accuracy, precision, specificity etc.

Linearity. Under the optimized experimental conditions, a linear relationship was observed between the relative fluorescence intensity recorded for each of NER and PEL at the selected wavelengths against the corresponding concentration within the linearity ranges stated in Table 1. The correlation coefficients, slopes, and intercepts obtained by the linear least squares regression analysis of the results were also calculated. The high values of the correlation coefficients (r-values >0.999) with small intercepts indicated good linearity of the calibration graphs. Other parameters including standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b) were also presented for each compound. ($S_{y/x}$) is required for the calculation of both (S_a) and (S_b) and measures the degree of deviation of the measured y-values from the calculated ones. The smaller the ($S_{y/x}$), the closer the points are to the linear regression line. Moreover, the variance ratio (F-values) were calculated. An increase in the variance ratio (F-values) indicates an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals, providing equal degrees of freedom. Thus high F-values indicate high steepness of the regression line along with low scatter of the experimental points around the regression line. Consequently, good regression lines show high values for both (r) and (F) values (29).

Limit of detection (LOD) and limit of quantitation (LOQ). LOD and LOQ were calculated for each of NER and PEL as per the ICH guidelines (28). The formulae used were (LOD = 3.3S/b) and (LOQ = 10S/b), where S is the standard deviation of blank determinations at the selected wavelengths and b is slope of the calibration curve. The calculated values of LOD and LOQ for both drugs were calculated as presented in Table 1.

Accuracy and precision. Accuracy and precision of the proposed methods were evaluated in terms of percentage relative error (E_r%) and percentage relative standard deviation (%RSD), respectively. They were validated by analyzing three solutions of each of NER and PEL at three different concentration levels within the linearity range of each compound (Table 2). Intra-day and inter-day accuracy and precision were assessed by repeating the assay three times on the same day or on three different days,

Table 2. Evaluation of the intra-day and inter-day accuracy and precision for the determination of NER and PEL by the proposed spectrofluorimetric method

Inhibitor	Concentration added (μg/mL)	Intra-day		Inter-day	
		Mean recovery (%) ± RSD	E_r(%)	Mean recovery (%) ± RSD[a]	E_r(%)
NER	10	99.20 ± 1.14	−0.80	100.01 ± 1.54	0.01
	1	98.64 ± 0.45	−1.36	99.85 ± 0.76	−0.15
	0.1	101.17 ± 1.36	1.17	98.46 ± 1.60	−1.54
PEL	10	98.51 ± 1.19	−1.49	99.64 ± 0.21	−0.36
	1	99.30 ± 1.93	−0.70	100.44 ± 1.66	0.44
	0.1	99.08 ± 1.61	−0.92	99.52 ± 0.61	−0.48

Mean recovery (%) ± relative standard deviation (RSD) of three determinations. E_r (%): percentage relative error.

respectively. The calculated errors and %RSD for both compounds were found to be less than 2%, indicating the high degree of accuracy and precision of the proposed methods (Table 2).

Stability of solutions. Standard solutions of NER and PEL (10 μg/mL) were found to be stable when left at room temperature for 8 h or stored in the refrigerator (4 °C) for 3 weeks. This was assessed by comparing the relative fluorescence intensity of tested solutions with freshly prepared solutions at the selected wavelengths. As the calculated errors were found to be <2%, this indicated the stability of NER and PEL standard solutions at room temperature for 8 h or at 4 °C for 3 weeks.

Robustness. The robustness of the proposed method was validated by making small deliberate changes in the experimental parameters. This aspect included changing in the pH of the phosphate buffer (10.5 ± 0.5). No significant change in the measured fluorescence intensity of the studied drugs at the selected wavelengths (%RSD < 2%) was noted, indicating high degree of method robustness.

Selectivity. The selectivity of the method was assessed by the analysis of the two drugs in mixtures with different excipients that could be found in actual tablets formulation (e.g. lactose, methyl cellulose, iron oxide, etc.). Good percentage recoveries with low errors and low %RSD were obtained following the analysis of both NER and PEL from laboratory-prepared mixtures with the added excipients indicating the absence of interference from such excipients. This ensures good selectivity of the proposed method.

Table 3. Determination of NER and PEL in laboratory-prepared tablets by the proposed spectrofluorimetric method

Mean recovery (%) ± RSD	
NER	98.20 ± 1.74
PEL	99.50 ± 1.07

Mean recovery (%) ± relative standard deviation (RSD) of five determinations.

Pharmaceutical analysis

The proposed method has been applied to the determination of NER and PEL in laboratory-prepared tablet mixtures. The results obtained from the proposed method revealed the ability of the method to determine both drugs in tablet formulations without any interference from co-formulation excipients, as indicated by the high degree of accuracy (% recovery) and precision (%RSD) (Table 3).

Forced-degradation studies

Forced-degradation studies can help identify possible degradation products of a drug substance, hence giving relevant information about the intrinsic stability of the compound. These studies are also important to identify the stability-indicating potential of the proposed analytical procedure (30,31). Standard solutions of NER and PEL were subjected to different stress conditions including strongly acidic (2 M hydrochloric acid solution), strongly basic (0.2 M sodium hydroxide solution), oxidative (0.5% hydrogen peroxide solution), and photolytic (direct daylight, UV radiation) degradation experiments.

No signs of degradation of both NER and PEL were observed in strongly acidic conditions, even following their heating in a boiling water bath with 2 M hydrochloric acid for 1 h. The same was recorded with photo-degradation under daylight (room temperature, 24 h) or using UV radiation (254 or 366 nm, 2 h). Also, no significant changes were recorded for both drugs following wet degradation studies (heating in a boiling water bath for 60 min). These findings indicated that both NER and PEL are stable towards acidic, photolytic, and wet degradation studies.

The situation was much different in the case of base-induced and oxidative degradation. Both NER and PEL showed apparent base-induced degradation. This degradation could be noticed by visual observation when a yellow-coloured solution that turns turbid after 30 min of boiling was noticed with PEL. While with NER, white turbidity was produced immediately after the addition of sodium hydroxide solution, which continued after boiling for up to 1 h. Boiling of PEL solutions with 2 M NaOH for 1 h resulted in nearly disappearance of the emission peak of PEL at 465 nm with

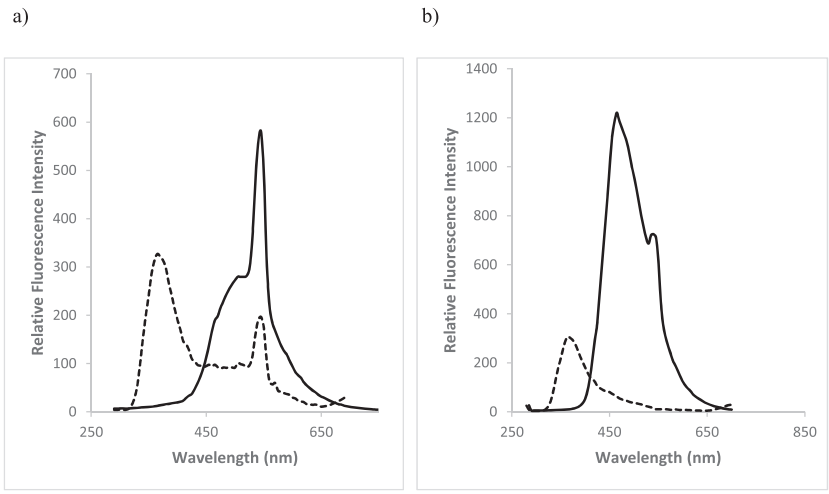

Figure 4. Emission fluorescence spectra of (a) neratinib (NER) 10 μg/mL; and (b) pelitinib (PEL) 10 μg/mL; in borate buffer pH 10.5 following base-induced degradation (boiling with 0.2 M sodium hydroxide for 60 min). Dotted spectra represent degraded samples while solid spectra represent intact samples.

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the formation of a new emission peak at 360 nm. Compared with PEL, NER was less susceptible to base degradation under the same conditions in which a new emission peak appeared at 360 nm without the disappearance of the drug peak at 545 nm with approximately 60% degradation (Fig. 4). Similarly, both NER and PEL were susceptible to oxidative degradation with 0.5% hydrogen peroxide. Moreover, heating NER and PEL solutions with 3% hydrogen peroxide at 90°C for 30 min resulted in a complete degradation of both drugs with disappearance of the emission peaks at the selected wavelengths of the intact drugs with no appearance of other emission peaks all over the spectra.

Degradation kinetics

The kinetics of base-induced degradation of NER and PEL were investigated using 0.2 M sodium hydroxide at different temperatures (70, 80, 90 and 100 °C) while oxidative degradation was carried out using 0.5% hydrogen peroxide solution at different temperatures (60, 70, 80 and 90 °C). As the decomposition rate of both drugs with either high strength sodium hydroxide (2 M or even 1 M) or hydrogen peroxide (3% or even 1%) was too rapid to obtain reliable kinetic data, solutions of 0.2 M sodium hydroxide or 0.5% hydrogen peroxide were used instead.

Base-induced degradation. A regular decrease in the concentration of both NER and PEL with increasing time intervals was observed. At different temperatures (70, 80, 90, 100 °C), the degradation process followed pseudo-first-order kinetics (Fig. 5a, b). Degradation rate constants (k) and half-lives ($t_{½}$) at each temperature (Table 4) were calculated from the slopes of the straight lines by the equation (32,33):

$$\log C = \log C^0 - kt/2.303 \quad (1)$$

where the slope of the line = − k/2.303 and C, C^0 are the drug concentrations measured at a given times t and at zero time, respectively.

Rate constants were then subjected to fitting in Arrhenius equation:

$$\log k = \frac{\log A - E_a}{2.303RT} \quad (2)$$

where A is the frequency factor, E_a is the activation energy (cal. mol^{-1}), R is the gas constant (1.987 cal/K mol) and T is the absolute temperature.

An Arrhenius plot was constructed by plotting the log of the rates of degradation against the reciprocals of the absolute temperature (32,33). The stability of the drugs at room temperature was estimated by extrapolating the rate constant at room temperature (k_{25}) (Fig. 5a′, b′). Degradation rate constants (half-lives) for NER and PEL were found to be 0.00061 min^{-1} (19.05 h), and 0.00610 min^{-1} (1.89 h), respectively. The results revealed that PEL is more susceptible to base-induced degradation, compared with NER.

It is noteworthy to mention that the pH-stability profile was previously generated for NER (34). It was reported that its degradation followed pseudo-first-order kinetics for all the pH's and temperatures studied suggesting that the rate was dependent on the protonation of the basic dimethylamino group within the NER sidechain. This report (34) proposed the mechanism involved in NER degradation showing that only the dimethylamino crotonamide part of NER was involved in the degradation in which allylamine–enamine isomerization is the initial and rate-limiting step in the degradation. This was followed by isomerization of

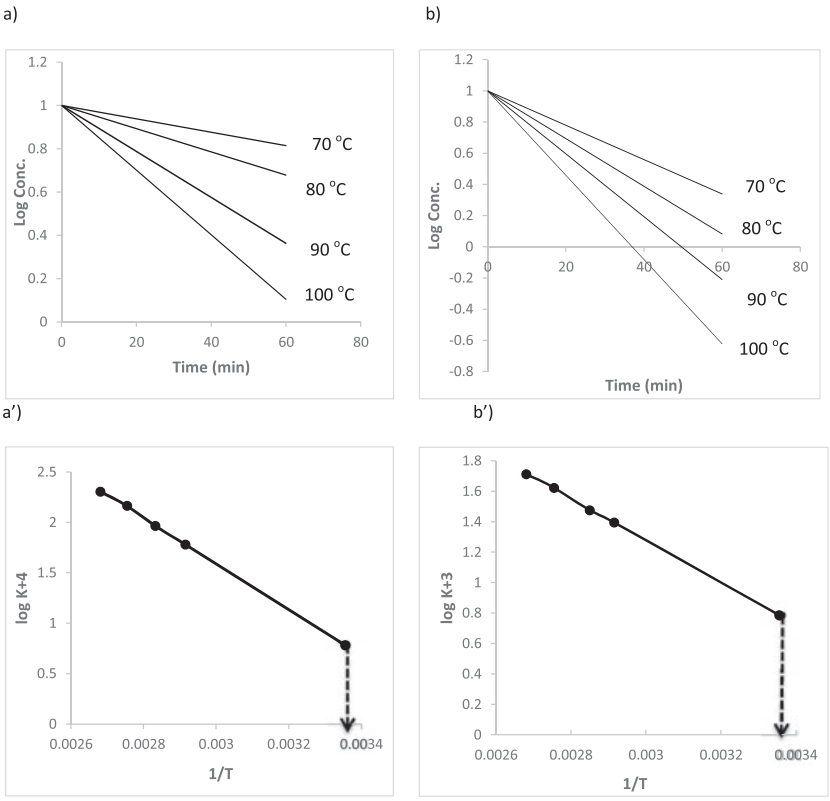

Figure 5. Kinetic study of the base-induced degradation of (a) neratinib (NER) 10 μg/mL; and (b) pelitinib (PEL) 10 μg/mL; at different temperatures, and their corresponding Arrhenius plots (a′) and (b′).

crotonamide to form enamine, which is then hydrolyzed to produce dimethylamine and aldehyde. The latter is then liable to intramolecular cyclization to form the five-membered lactam product, which is the final degradation product reported for NER. The same degradation scheme could be suggested for PEL degradation as it has the same dimethylamino crotonamide side chain. The new emission peak appearing at 360 nm in base-induced degraded samples for both drugs is most probably due to the formation of the cyclized lactam product with suggestion of the formation of the same degradation product for both NER and PEL. As this report (34) suggested that the free basic form is reactive, while the protonated form is unreactive, this aspect could explain the stability of both NER and PEL towards acidic degradation.

Table 4. Pseudo-first-order rate constants (k) and half-lives ($t_{½}$) for base-induced and oxidative degradation of NER and PEL at different temperatures

Inhibitor	Temperature (°C)	Base-induced degradation k (min^{-1})	$t_{½}$ (min)
NER	100	0.020	34.46
	90	0.015	47.67
	80	0.009	75.36
	70	0.006	115.10
PEL	100	0.051	13.47
	90	0.042	16.53
	80	0.030	23.20
	70	0.025	27.93
Inhibitor	Temperature (°C)	Oxidative degradation k (min^{-1})	$t_{½}$ (min)
NER	90	0.056	12.33
	80	0.046	15.16
	70	0.025	27.70
	60	0.010	68.87
PEL	90	0.078	8.87
	80	0.070	9.77
	70	0.031	22.08
	60	0.015	45.05

Oxidative degradation. The effect of hydrogen peroxide on the degradation of both NER and PEL was studied. To study the degradation kinetics, 0.5% solution of hydrogen peroxide was used at different temperatures. Plotting the logarithmic concentration of drug remaining as a function of heating time at different temperatures (60, 70, 80 or 90 °C) revealed that the degradation process followed pseudo-first-order kinetics (Fig. 6a, b).

The pseudo-first-order rate constants and half-lives were calculated based on eqns 1 and 2, respectively (Table 4). As under base-induced degradation, Arrhenius plot was constructed and the stability of the drugs at room temperature was estimated (Fig. 6a′, b′). Accordingly, the rate constant at room temperature (k_{25}) was found to be 0.0010 and 0.00146 for NER and PEL, respectively. The half-lives required for causing 50% degradation of NER and PEL at room temperature were 11.55 and 7.91 h, respectively. As in base-induced degradation, PEL is more susceptible to oxidative degradation, compared with NER.

As oxidative degradation of both NER and PEL resulted in a significant decrease in the emission peaks at the selected wavelengths without the appearance of other emission peaks, it is

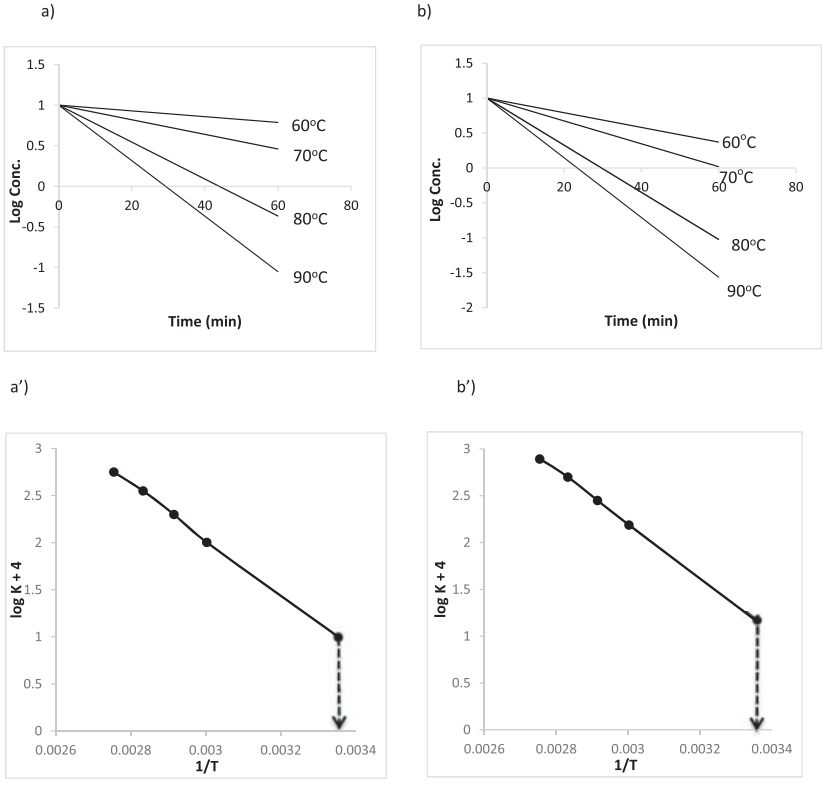

Figure 6. Kinetic study of the oxidative degradation of (a) neratinib (NER) 10 μg/mL; and (b) pelitinib (PEL) 10 μg/mL; at different temperatures, and their corresponding Arrhenius plot (a′) and (b′).

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postulated that oxidative degradation does not follow the same degradation mechanism as base-induced degradation. Most likely the oxidative degradation product has much less fluorescence quantum yield compared with the parent drug. Both drugs could be affected by hydrogen peroxide in different ways; formation of epoxy derivatives on the alkene side chain, or even N-oxides or further cleavage of the quinoline ring (35,36).

Conclusion

The proposed emission fluorimetric method depends on the direct measurement of the relative fluorescence intensity of NER and PEL at the selected wavelengths. Moreover, this work studies – for the first time – the intrinsic fluorescence of both drugs under different experimental conditions, indicating its possible applicability for the simple and direct determination of the drugs in bulk powders or any possible future pharmaceutical preparations. Additionally, the proposed method has been adapted for stability studies of NER and PEL as the first published stability-indicating method. The speed of analysis, minimum sample preparation, and low cost are the main advantages of the direct fluorimetry compared with the more sophisticated LC–MS/MS chromatographic techniques reported for the determination of either NER (10–12,14) or PEL (15,16). Moreover, the emission spectra of the studied drugs were simply measured in borate buffer solution (pH 10.5) without the need of any organic solvents. Thus it has an additional advantage of being a non-hazardous eco-friendly method.

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