DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF GEFITINIB IN BULK AND IN FORMULATION

S. R. Patra, A. Bali*, M. Saha

University Institute of Pharmaceutical Sciences, UGC Center of Advanced Study, Panjab University, Chandigarh, 160014, India; e-mail: alka.bali@rediffmail.com

A rapid, sensitive, cost effective and reproducible first-order derivative spectrophotometric method was developed for the estimation of gefitinib in bulk and in its marketed formulation. Preliminary spectrophotometric determination of the drug was carried out in acetate buffer pH 2.8 and in 0.1 N HCl with a total of 20 parametric variations. The selected method with three parametric variations employing peak-zero (P-0) and peak-peak (P-P) techniques was assessed for stability indicating potential in force degraded solutions. The developed method was validated with respect to linearity, accuracy, precision, and robustness. Linearity was observed in the concentration range of 5–50 μg/ml with an excellent correlation coefficient (r^2) of 0.999. The limits of assay detection values were found for the range from 1.69–3.75 μg/ml, and quantitation limits ranged from 5.11–12.71 μg/ml for the proposed method. The proposed method was applicable for the determination of the drug in its marketed tablet formulation, and percentage recovery was found for the range from 97.42 to 98.58%.

Keywords: *gefitinib, derivative spectrophotometry, method validation, analysis.*

МЕТОДЫ ПРОИЗВОДНОЙ СПЕКТРОФОТОМЕТРИИ ДЛЯ ОПРЕДЕЛЕНИЯ ГЕФИТИНИБА В НЕФАСОВАННОЙ И ФАРМАЦЕВТИЧЕСКОЙ ФОРМАХ

S. R. Patra, A. Bali*, M. Saha

УДК 543.42.062

Институт фармацевтических наук Центра перспективных исследований UGC Пенджабского университета, Чандигарх, 160014, Индия; e-mail: alka.bali@rediffmail.com

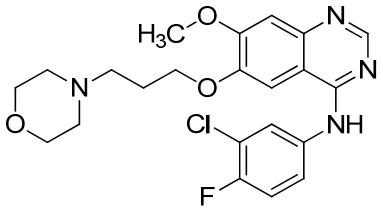
(Поступила 21 августа 2020)

Разработан быстрый, чувствительный, рентабельный и воспроизводимый спектрофотометрический метод производной первого порядка для определения гефитиниба в объеме вещества, а также в формах, поступающих на рынок. Предварительное спектрофотометрическое определение препарата проведено в ацетатном буферном веществе с pH 2.8 и в 0.1 N HCl с 20 вариантами параметров. Выбранный метод с тремя вариантами параметров на основе методов пик-ноль (P-0) и пик-пик (P-P) оценен на стабильность, указывающую на возможность его применения для растворов с ускоренной деградацией. Разработанный метод апробирован на предмет линейности, точности и устойчивости. Линейность наблюдалась в диапазоне концентраций 5–50 мкг/мл с коэффициентом корреляции r^2 = 0.999. Пределы детектирования наблюдались в диапазоне 1.69–3.75 мкг/мл, а пределы количественного определения 5.11–12.71 мкг/мл. Предложенный метод применим для определения лекарственного средства в составе имеющихся в продаже таблеток с вероятностью обнаружения 97.42–98.58 %.

Ключевые слова: *гефитиниб, производная спектрофотометрия, валидация метода, анализ.*

Introduction. Gefitinib (GEF) (Iressa® by AstraZeneca; Gefticip® by Cipla Ltd.) (CAS Number: 184475-35-2) (N-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-(morpholin-4-yl)propoxy)quinazolin-4-amine)

is approved by the U.S. FDA as a first-line drug for treatment of patients with metastatic non-small-cell lung carcinoma (NSCLC) [1, 2]. The drug is mechanistically an inhibitor of tyrosine kinase of epidermal growth factor receptor (EGFR).



Gefitinib

Survey of the available literature on gefitinib revealed some reports on the analytical method development for the drug. Most of them involved the application of chromatographic techniques for the estimation of gefitinib in bulk and in pharmaceutical dosage forms [3, 4]. A validated rapid resolution liquid chromatographic method was reported for determination of gefitinib in the presence of two of its impurities [5]. Several chromatographic studies relating to the clinical determination of gefitinib are reported. They include the HPLC-UV method [6] for estimation of gefitinib and erlotinib in human plasma, a variety of LC/MS/MS based methods for the determination of GEF in the presence of other tyrosine kinase inhibitors (TKIs) in biological samples including plasma/blood samples [7–12], and determination of gefitinib in the presence of its major metabolites by LC-UV and LC-MS techniques [13–16]. Further, some zero-order UV/visible spectrophotometric methods were reported in the literature for the quantitative estimation of the drug [17–21]. Derivative spectrophotometry is a versatile technique that offers several advantages over simple zero-order spectrophotometry in terms of increased sensitivity and selectivity. Analysis of the literature reports suggested that the mentioned spectrophotometric studies were not comprehensive enough to fully explore the scope of the method as zero-order and higher-order derivative techniques. Hence, the present investigation describes a simple, reproducible derivative spectrophotometric method for quantification of gefitinib in bulk and in its tablet formulation.

Experiment. *Chemicals and reagents.* Gefitinib (Batch number IWC170066) was graciously provided as a gift sample by CIPLA Pharma Ltd, Mumbai (India). Analytical reagent (AR) grade chemicals and materials were purchased from Merck Life Sciences (Mumbai, India) and were employed throughout the study. All solutions were freshly prepared using triple distilled water obtained from Milli-Q plus purification system Millipore (Bradford, USA). All absorption spectra were recorded using a Perkin Elmer lambda 35 UV-visible spectrophotometer with a scanning speed of 60 nm/min, spectral slit width of 2.0 nm, and resolution of 2.0 nm, equipped with 10 mm matched quartz cells. Digital pH meter (Eutech Instruments, model GC7252101B) was used to adjust the pH of the buffer solution. Geftib® tablets (label amount is 250 mg gefitinib per tablet, Glenmark Pharmaceutical Ltd.) were purchased from a market. All the glassware including volumetric flasks, beakers, pipettes, measuring cylinders, round bottom flasks were Class A apparatus from Borosil.

Sample preparation and analysis. Standard stock solution (1000 μg/ml) of gefitinib in acetate buffer pH 2.8 (4.0 g of anhydrous sodium acetate dissolved in triple distilled water; pH adjusted with glacial acetic acid and final volume made up to 1000 ml) or 0.1 N HCl was prepared every day, and this was diluted (1 in 10) to obtain the stock solution (100 μg/ml). Serial dilutions of the stock solution were carried out with appropriate solvents (acetate buffer pH 2.8 or 0.1 N HCl) to obtain the working standard solutions (0.5 to 80 μg/ml). Zero-order and first-order derivative spectra were recorded over the wavelength range of 210–400 nm against reagent blank, and absorbance values (zero-order spectra) or amplitudes of the maximum and minimum (first-order spectra) were measured.

Experimental method validation. Two spectrophotometric methods A and B (in acetate buffer pH 2.8 and 0.1 N HCl, respectively) were performed with a total of 20 parametric variations, and three optimized variants of the method B were validated with respect to various parameters outlined in the ICH guideline Q2 (R1).

The working standard solution (100.0 μg/ml) was serially diluted with an appropriate reagent (acetate buffer pH 2.8 or 0.1 N HCl) to prepare solutions with concentrations ranging from 0.5–80.0 μg/ml (0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40.0, 60.0, and 80.0 μg/ml) of the drug. All these dilutions along with the working standard solution, prepared in triplicate, were analyzed by various zero-order and first-order spectrophotometric methods.

The intraday precision of the methods (selected based on linearity studies) was determined by the analysis of three varying concentrations of the drug (5.0, 10.0, and 20.0 μg/ml) on a single day. Determination of interday precision was carried out by analyzing three samples of varying concentrations on 3 successive days. The precision was expressed as RSD% corresponding to each calculated concentration of the analyte.

A preanalyzed solution of the pure drug gefitinib was suitably diluted to obtain the unspiked solution of the drug (10.0 μg/ml) for accuracy analysis. This solution was then spiked by 50, 100, and 150% to provide concentration increases by 5.0, 10.0, and 15.0 μg/ml, respectively, by mixing the unfortified solution separately with equal volumes of the standard drug solutions of strengths of 20.0, 30.0, and 40.0 μg/ml, respectively. The drug concentration in the fortified solutions (final analyzed concentrations of 15.0, 20.0, and 25.0 μg/ml) and the unfortified solution were then determined (n = 3). Method accuracy was expressed as percent recovery of the fortified drug concentration with reference to the unfortified one.

Robustness was assessed by carrying out deliberate changes in the method variables, including temperature and buffer pH, and studying their impact on the recovery of the drug in the test solutions.

Analysis of marketed formulation (Gefitinib tablets). A weighed portion of the powder, equivalent to 100 mg of gefitinib (Geftib® tablets; label amount 250 mg gefitinib per tablet, Glenmark Pharmaceutical Ltd.), was dissolved in an appropriate reagent (acetate buffer pH 2.8 or 0.1 N HCl) to prepare 100 ml of the solution A (1000 μg/mL). The solution was suitably diluted and analyzed for the drug content by three variants of the developed method B.

Results and discussion. Derivative spectrophotometry offers significant advantages over zero-order spectrophotometry in terms of enhanced specificity and selectivity. The selection of appropriate peak amplitudes in the derivative curves can permit drug analysis in the presence of excipients, degradation products. and other impurities. In this light, a comprehensive study was carried out to thoroughly explore all possible zero- and first-order derivative spectrophotometric curves of gefitinib to develop a sensitive and reproducible stability-indicating method for the drug.

Method development. A preliminary analysis of UV absorption and solubility characteristics of the drug was carried out to select an appropriate buffer/solvent system for the development of the method. Experimental log P of the drug is 3.2, and its experimental pK_α values are 5.4 and 7.2. Gefitinib has a low aqueous solubility (0.027 mg/ml), and the solubility of the drug is strongly dependent on pH, as the drug is sparingly soluble at pH 1. Further growth in pH increases its solubility, but the solubility drops sharply between pH range of 4 to 6, and the drug becomes practically insoluble above pH of 7. Further, gefitinib is slightly soluble in UV-transparent nonaqueous solvents such as methanol and ethanol (99.5%) (http://drugbank.ca/drugs/DB00317). Based on this data, acetate buffer pH 2.8 and 0.1 N HCl were selected for the spectrophotometric method development and validation. The zero- and first-order derivative spectra for the standard solutions of gefitinib ranging from 0.5 to 80.0 μg/ml were recorded over the wavelength range of 210–400 nm, taking acetate buffer pH 2.8 or 0.1 N HCl as a reagent blank. The amplitudes of the maxima and minima were measured for all derivative spectra. Figures 1 and 2 depict the zero-order and first-order derivative UV overlay spectra of gefitinib in acetate buffer pH 2.8 and 0.1 N HCl, respectively.

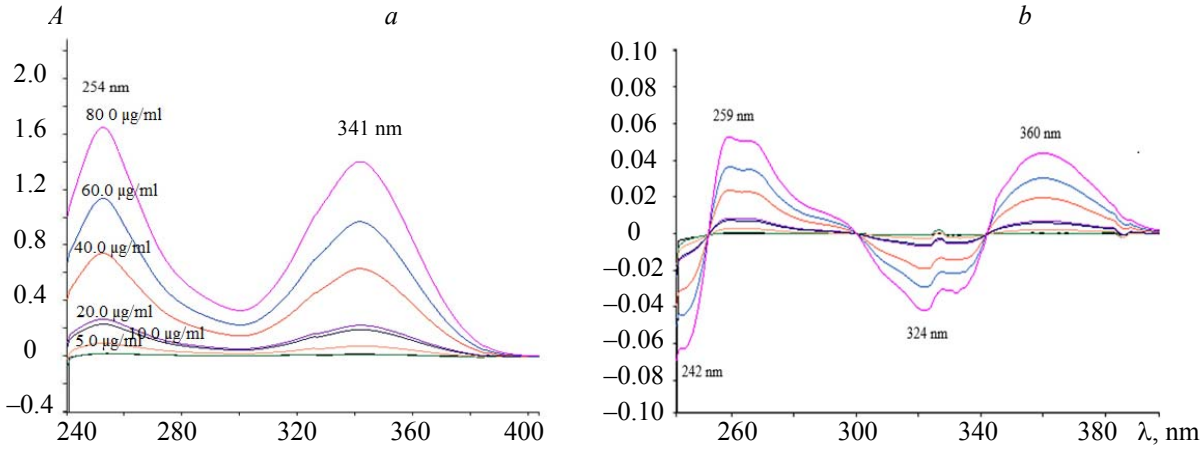


Fig. 1. Zero-order (a) and first-order (b) derivative overlay UV spectra of gefitinib in acetate buffer pH 2.8 (5.0, 10.0, 20.0, 40.0, 60.0, and 80.0 μg/ml).

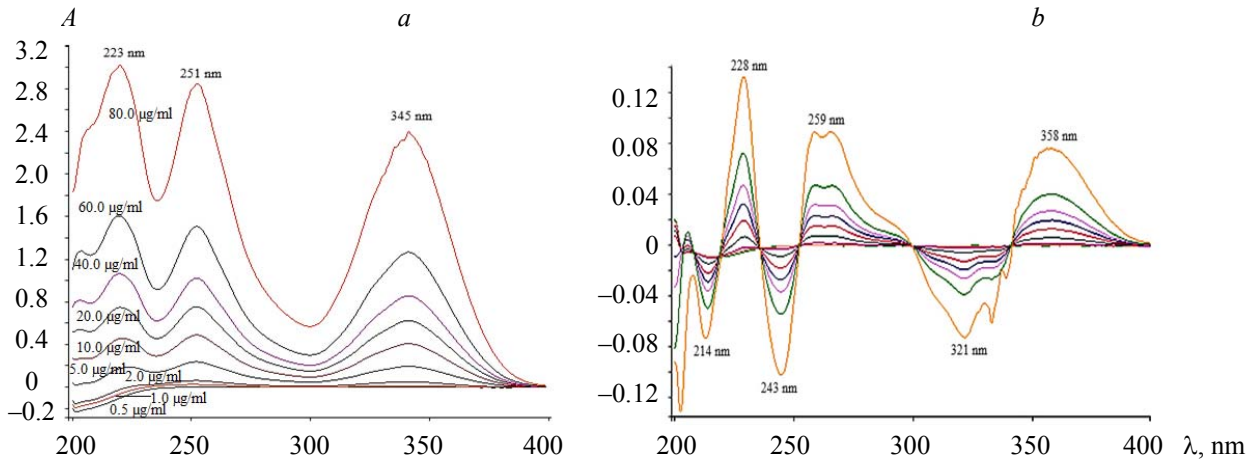


Fig. 2. Zero-order (a) and first-order (b) derivative overlay UV spectra of gefitinib in acetate 0.1 N HCl (0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40.0, 60.0, and 80.0 µg/ml).

Calibration curves of Gefitinib. The regression parameters, Beer's law limits, and wavelength range for the working standard solutions of gefitinib employing 20 variants (zero-order and first-order) of methods A and B are summarized in Table 1. Two zero-order and six first-order derivative UV spectrophotometric variants were studied for method A (in acetate buffer pH 2.8), whereas, three zero-order and nine first-order derivative UV spectrophotometric variants were studied for method B (in 0.1 N HCl). Among them, three variants of method B (12, 13, and 14) were selected for further validation as peak amplitudes (zero or peak-to-peak) afforded the best linear correlation in these methods. Figure 3 shows the standard plots of gefitinib with the selected methods.

TABLE 1. Linearity and Range for the Explored Methods for Analysis of Gefitinib by Zero-order and First-order Derivative Spectrophotometry

Method type	Method variant	Beer's law limits, µg/ml	λ, nm	Technique	Regression equation	r^2
A[a]	Zero order	1–30	254	Abs	$y = 0.0562x - 0.0391$	0.9953
A	Zero order	1–30	341	Abs	$y = 0.0481x - 0.0344$	0.9962
B[b]	Zero order	5–50	223	Abs	$y = 0.0628x - 0.1765$	0.9971
B	Zero order	1–30	251	Abs	$y = 0.0566x - 0.0688$	0.9958
B	Zero order	1–30	345	Abs	$y = 0.0479x - 0.0654$	0.9955
A	First order	2–30	242	P–0	$y = 0.0010x + 0.0140$	0.9670
A	First order	2–30	259	P–0	$y = 0.0018x - 0.0005$	0.9602
A	First order	2–30	242–269	P–P	$y = 0.0030x + 0.0140$	0.9700
A	First order	2–30	324	P–0	$y = 0.0010x + 0.0010$	0.9690
A	First order	2–30	360	P–0	$y = 0.0010x + 0.0010$	0.9670
A	First order	2–30	324–360	P–P	$y = 0.0020x + 0.0010$	0.9700
B	First order	5–50	214	P–0	$y = 0.0014x + 0.0074$	0.9993
B	First order	5–50	228	P–0	$y = 0.0025x - 0.0047$	0.9993
B	First order	5–50	214–228	P–P	$y = 0.0039x + 0.0027$	0.9994
B	First order	2–30	243	P–0	$y = 0.0020x - 0.0032$	0.9951
B	First order	2–30	259	P–0	$y = 0.0018x - 0.0026$	0.9959
B	First order	2–30	243–259	P–P	$y = 0.0038x - 0.0058$	0.9955
B	First order	2–30	321	P–0	$y = 0.0015x - 0.0024$	0.9952
B	First order	2–30	358	P–0	$y = 0.0015x - 0.0024$	0.9958
B	First order	2–30	321–358	P–P	$y = 0.0030x - 0.0048$	0.9955

[a] Calibration data in acetate buffer pH 2.8.
[b] Calibration data in 0.1 N HCl.

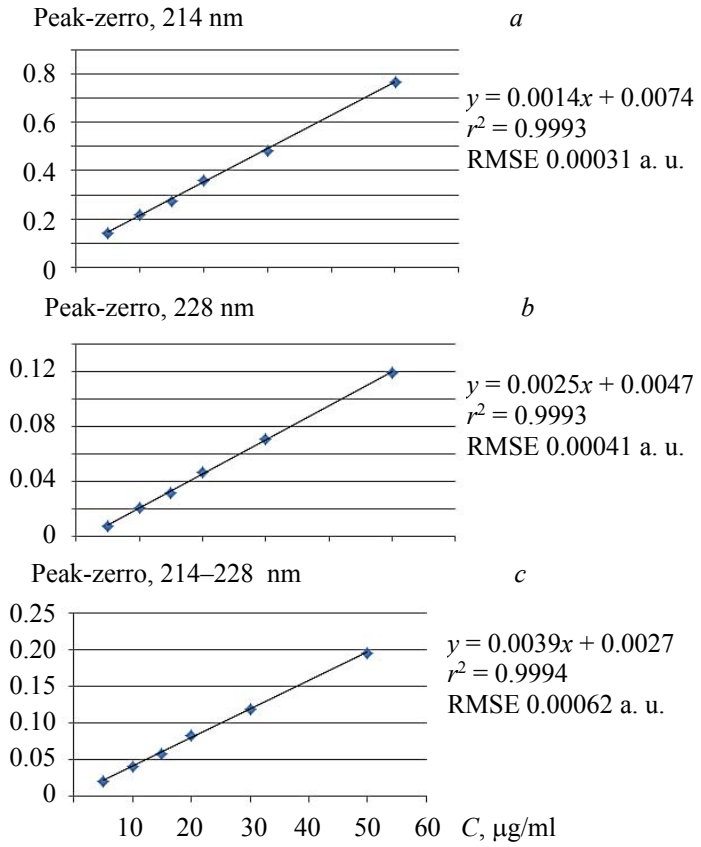


Fig. 3. Standard plots of gefitinib with method variants 12 (a), 13 (b), and 14 (c).

The method was validated with respect to linearity and range, accuracy and precision, and limit of detection (LOD) and limit of quantification (LOQ). The various method validation parameters are summarized in Tables 1–3.

The absorbance measurements (zero-order spectra) and the peak-to-zero (P-0) or peak-to-peak (P-P) amplitude measurements (first-order derivative spectra) were done at varying wavelengths in the concentration range of 0.5–80.0 μg/ml of gefitinib. The various regression parameters corresponding to the different variants of methods A and B are summarized in Table 1. Values of the correlation coefficient r^2 were found to be above 0.9 in many cases, indicating good linearity over the working concentration ranges. Method variants delivering the best r^2 values, i.e., close to 1.0, were selected for further analytical validation (12, 13, and 14). Excellent compliance with the Beer–Lambert law (linearity) was noted in the concentration range of 5.0–60.0 μg/ml, for all of three methods selected. RMSE values were found to be 0.00031, 0.00041, and 0.00062 amplitude units (a. u.), respectively, for method variants 12, 13, and 14.

LOD and LOQ of the method were established using calibration standards. LOD and LOQ were calculated as 3.3σ/s and 10σ/s, respectively, as per ICH definitions, where σ is the mean standard deviation of replicate determination values under the same conditions as the sample analysis in the absence of the analyte (blank determination), and s is the sensitivity, namely, the slope of the calibration graphs. LOD and LOQ values for all method variants 12, 13, and 14 were found to be 2.75 and 5.36, 2.19 and 5.71, and 1.69 and 5.11 μg/ml, respectively.

Precision was investigated by analyzing three different concentrations of gefitinib (5.0, 10.0, and 20.0 μg/ml) in three independent repeats on the same day (to evaluate intraday precision) and on three consecutive days (to evaluate interday precision). These intraday and interday precision data, represented as relative standard deviation (RSD%), are shown in Table 2. The RSD% values in the intraday and the interday precision study were found to be less than 2.49 and 1.95%, respectively, for method variants 12, 13, and 14 indicating good precision of the method.

TABLE 2. Validation Parameters for the Proposed Method

Parameter	Gefitinib					
Accuracy	Concentration (μg/ml) ± S.D.; RSD%[#]					
Concn. of drug taken, μg/ml	Spiked drug conc. μg/ml (%)[*]	Calculated[**] % Recovery by method variants				
		12	13	14		
10.0	5.0 (50%)	98.19±0.0002 0.85	94.47±0.00015 0.49	91.96±0.00046 0.98		
10.0	10.0 (100%)	105.27±0.0002 0.42	102.60±0.00068 1.39	109.73±0.00125 1.79		
10.0	25.0 (150%)	95.87±0.0006 1.45	93.33±0.00085 1.49	107.08±0.00072 0.86		
Precision	Peak amplitude[**] ± S.D.; RSD%[#] with method variants 12, 13, and 14					
Conc. taken, μg/ml	Intraday (n = 6)			Interday (n = 3)		
	12	13	14	12	13	14
5.0	0.01±0.0001; 0.95	0.01±0.0001; 1.50	0.02±0.0005; 1.49	0.01±0.0001; 1.29	0.01±0.0006; 0.81	0.02±0.0004; 1.67
10.0	0.02±0.0004; 1.79	0.02±0.0004; 1.88	0.04±0.0006; 1.53	0.02±0.0003; 1.48	0.02±0.0004; 1.80	0.04±0.0007; 1.59
20.0	0.04±0.0006; 1.71	0.05±0.0006; 1.18	0.07±0.0012; 1.70	0.04±0.0004; 1.12	0.05±0.0009; 1.96	0.07±0.0006; 0.85
LOD, μg/ml (method number)	2.75 (12); 2.19 (13); 1.69 (14)					
LOQ, μg/ml (method number)	5.36 (12); 5.71 (13); 5.11 (14)					

[*]Equal volumes of drug solutions (20, 30, and 40 μg/ml) added to preanalyzed drug solution (10 μg/ml).
[**]Calculated as a mean of measurements in triplicate (n = 3).
[#]Calculated as: SD/mean ×100.

The accuracy of the proposed methods was assessed by preparing different concentration levels of drug for analysis from independent stock solutions. Further assessment of accuracy of the developed methods was carried out by spiking excess drug (50, 100, and 150%) to preanalyzed drug solution samples (10 μg/ml). Accuracy was determined as mean % recovery and RSD%. Excellent recovery values for method variants 12, 13, and 14 ranging from 91.96–109.73% (Table 2) indicated good accuracy of the method.

Repeatability of an analytical method is assessed from robustness, which is examined by evaluating the effect of small variances in experimental conditions such as heating temperatures (±2°C). Three replicate determinations at six different concentration levels of the drugs were carried out at ambient temperature (26°C) and at 28 and 23°C (room temperature ±2°C). The intraday RSD values for the method variants 12, 13, and 14 were found to be less than 1.0%, indicating that the proposed method variants have reasonable robustness.

The stability of the final sample solutions was examined by their absorbance values/peak amplitudes, and responses were found to be stable for at least 8 h at room temperature.

Table 3 shows the results of the assay for gefitinib carried out on the marketed formulation by three proposed method variants. The percentage recovery was found for the range from 97.42–98.58% (amount per tablet found to be 243.56–246.44 mg), which shows close agreement between the results obtained by the proposed method variants and the label claim.

TABLE 3. Recovery Studies from Marketed Drug Formulation

Method variant	Label claim, mg	Mean recovery (mg) ± SD[*]	Mean recovery± SD[*]%	RSD, %
12	250	243.56 ± 2.45	97.42 ± 0.98	1.01
13	250	246.44 ± 3.35	98.58 ± 1.35	1.37
14	250	244.90 ± 2.85	97.96 ± 1.14	1.16

[*]Average of three determinations.

Conclusions. Three variants of a rapid, sensitive, inexpensive and accurate first-derivative method were developed for quantification of gefitinib in bulk as well as in its marketed formulation (tablets). The method variants were validated in terms of their sensitivity, reproducibility, precision, accuracy, robustness, and solution stability for ≥8 h, suggesting their suitability for routine analysis of GEF in pure form (bulk analysis) as well as in pharmaceutical formulations, without interference from excipients. Excellent recovery of the drug from its force degraded solutions suggests the stability-indicating nature of the method and its potential applicability in the presence of routine degradation products. We have explored all wavelength regions in the zero-order and first-order derivative spectra of gefitinib for its estimation, and this has not been reported in previous studies. The validation parameters were found to be the best for method variants 12, 13, and 14. These methods can be explored further for analysis of gefitinib in other formulations containing varied excipients.

Acknowledgments. We sincerely thank CIPLA Pharma Ltd., Mumbai (India) for graciously providing pure drug samples of Gefitinib. The research grant provided by the University Grants Commission is duly acknowledged. We acknowledge the contribution of Sophisticated Analytical Instrumentation Facility, CIL and UCIM, Panjab University, Chandigarh, India for conducting LC-MS/TOF analysis.

REFERENCES

1. J. Y. Douillard, G. Ostoros, M. Cobo, T. Ciuleanu, R. McCormack, A. Webster, T. Milenkova, *Br. J. Cancer*, **110**, 55–62 (2014).
2. M. Fukuoka. Y. L. Wu, S. Thongprasert, P. Sunpaweravong, S. S. Leong, V. Sriuranpong, T. Y. Chao, K. Nakagawa, D. T. Chu, N. Saijo, E. L. Duffield, *J. Clin. Oncol*., **29**, 2866–2874 (2011).
3. A. Raja, P. Joshna, D. Banji, K. Rao, S. Duraipandi, *Asian J. Pharm. Anal. Med. Chem*., **2**, No. 3, 127–133 (2014).
4. A. Sreedevi, A. L. Rao, L. Kalyani, *Int. J. Pharm. Chem. Biol. Sci*., **3**, No. 4, 1305–1315 (2013).
5. M. Venkataramanna, I. V. Somaraju, K. S. Babu, *Am. J. Anal. Chem*., **2**, 75–83 (2011).
6. L. Faivre, C. Gomo, O. Mir, F. Taieb, A. Schoemann-Thomas, S. Ropert, M. Vidal, D. Dusser, A. Dauphin, F. Goldwasser, B. Blanchet, *J. Chromatogr. B*, **879**, 2345–2350 (2011).
7. G. Guetens, H. Prenen, G. De Boeck, W. Van Dongen, E. Esmans, F. Lemiere, A. T. Van Oosterom, P. Schöffski, E. A. De Bruijn, *J. Chromatogr. A*, **1082**, 2–5 (2005).
8. M. Zhao, C. Hartke, A. Jimeno, J. Li, P. He, Y. Zabelina, M. Hidalgo, S. D. Baker, *J. Chromatogr. B*, **819**, 73–80 (2005).
9. K. Irie, S. Shobu, S. Hiratsuji, Y. Yamasaki, S. Nanjo, C. Kokan, A. Hata, R. Kaji, K. Masago, S. Fujita, Y. Okada, *J. Chromatogr. B*., **1087**, 1–5 (2018).
10. A. Chahbouni, J. C. G. den Burger, R. M. Vos, A. Sinjewel, A. J. Wilhelm, *Ther. Drug Monit*., **31**, 683–687 (2009).
11. S. Bouchet, E. Chauzit, D. Ducint, N. Castaing, M. Canal-Raffin, N. Moore, K. Titier, M. Molimard, *Clin. Chim. Acta*, **412**, 1060–1067 (2011).
12. R. Honeywell, K. Yarzadah, E. Giovannetti, N. Losekoot, E. F. Smit, M. Walraven, J. S. Lind, C. Tibaldi, H. M. Verheul, G. J. Peters, *J. Chromatogr. B*, **878**, 1059–1068 (2010).
13. D. McKillop, M. Hutchison, E. A. Partridge, N. Bushby, C. M. Cooper, J. A. Clarkson-Jones, W. Herron, H. C. Swaisland, *Xenobiotica*, **34**, 917–934 (2004).
14. C. Karunakara, A. Udupi, C. G. Reddy, *J. Liq. Chromatogr. Relat. Technol*., **38**, 759–764 (2015).
15. N. Zheng, C. Zhao, X. R. He, S. T. Jiang, S. Y. Han, G. B. Xu, P. P. Li, *J. Chromatogr. B*, **1011**, 215–222 (2016).
16. L. Z. Wang, M. Y. Lim, T. M. Chin, W. L. Thuya, P. L. Nye, A. Wong, S. Y. Chan, B. C. Goh, P. C. Ho, *J. Chromatogr. B*, **879**, 2155–2161 (2011).
17. D. G. Sankar, B. A. Kumar, P. J. Babu, P. V. M. Latha, *Asian J. Chem*., **18**, No. 4, 3249–3250 (2006).
18. A. Ratnakumari, A. Rajeswari, K. Sobha, *Pharmanest*, **1**, No. 1, 118–122 (2010).
19. S. K. Savale, *Asian J. Biomater. Res*., **4**, No. 2, 18–22 (2017).
20. V. K. Redasani, P. S. Patel, S. J. Surana, *Anal. Chem*., **14**, No. 3, 83–87 (2014).
21. M. B. Varanasi, M. A. Khan, V. R. Jangala, B. B. Teja, *Int. J. Chem. Sci*., **7**, No. 4, 2549–2554 (2009).