Research Article

Hassan A. Alhazmi*, AbdulRhman Ali Bokar Nasib, Yasser Ali Musleh, Khaled Qassim Hijri, Zia ur Rehman, Gulrana Khuwaja, Mohammed Al-Bratty, Sadique A. Javed, Ismail A. Arbab

Application of drug–metal ion interaction principle in conductometric determination of imatinib, sorafenib, gefitinib and bosutinib

<https://doi.org/10.1515/chem-2020-0123>

received November 29, 2019; accepted May 18, 2020

Abstract: An analytical method for the quantification of anticancer agents such as imatinib, sorafenib, gefitinib and bosutinib using conductometry was developed. Each drug solution was mixed with measured concentration of metal ion (Cu^{2+}) solution resulting in drug–metal ion complexation in the titration cell. Conductance was progressively decreased on addition of the analyte solution up to a point of maximum reduction, that is, the end point. Corrected conductance values were calculated from the observed conductance and used to plot a graph against the volume of drug solution added. No interferences were observed from blank and placebo as they gave no clear inflection in the conductivity during titration. The precision and the accuracy of the developed method was established by the analysis of quality control samples; %RSD of corrected conductance values $<2\%$ and recovery results within $100 \pm 2\%$ were achieved. The calibration graphs obtained were linear over the concentrations 1.0–1.4 mM for all the drugs ($R^2 > 0.99$). The drugs were successfully analyzed in their respective dosage forms prepared in-house. The method has offered easier, faster and cost-effective analysis of the selected drugs and can be used for routine determinations in the quality control

laboratories. More importantly, it is an environmental friendly procedure, as no organic solvent was used throughout the analysis.

Keywords: bosutinib, conductometric analysis, gefitinib, imatinib, sorafenib

1 Introduction

Tyrosine kinase (TK) is one of the more than 500 members of protein kinase enzyme family, which play a pivotal role in various cellular processes such as proliferation, apoptosis, immune response and cell development. The dysregulation of TK may result in pathological conditions including cancer, and in the last few decades, TK inhibitors have emerged as one of the most studied drugs for cancer therapy [1–3]. Imatinib is the first clinically successful TK inhibitor mainly used in the treatment of chronic myeloid leukemia (CML). It is believed to act through inhibition of BCR-ABL protein kinase that is responsible for the regular proliferation of the myeloid cells [4]. Following the development of imatinib, more than 30 protein kinase inhibitors have been approved for clinical use in the treatment of different types of cancers including lung, kidney, blood, gastrointestinal tract, breast and skin. Other important TK inhibitors such as sorafenib, gefitinib, sunitinib and bosutinib are mainly used for the treatment of clear cell renal carcinoma, advanced non-small cell lung cancer, metastatic renal cell cancer and chronic myelogenous leukemia, respectively [5–7].

The literature survey revealed several analytical procedures that have been developed for estimation of TK inhibitors either as alone or in combinations. These methods were mainly based on the chromatographic techniques such as HPLC and LC-MS/MS [8–12]. These techniques are expensive and involve complex and time-consuming experimental procedures, in addition to the

* **Corresponding author: Hassan A. Alhazmi**, Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, Jazan, Post code 45142, Saudi Arabia; Substance Abuse and Toxicology Research Centre, Jazan University, P.O. Box 114, 45142, Jazan, Saudi Arabia, e-mail: haalhazmi@jazanu.edu.sa, tel: +966-541433344

AbdulRhman Ali Bokar Nasib, Yasser Ali Musleh, Khaled Qassim Hijri, Zia ur Rehman, Gulrana Khuwaja, Mohammed Al-Bratty, Sadique A. Javed: Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, Jazan, Post code 45142, Saudi Arabia
Ismail A. Arbab: Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, Jazan, Post code 45142, Saudi Arabia; Department of Chemistry, Faculty of Education, University of West Kordufan, West Kordufan State, El Nahud, 55511, Sudan

use of large amounts of hazardous solvents and high equipment cost. Therefore, the analytical methods based on these techniques are less preferred for the routine analysis in the quality control laboratories. Nevertheless, the use of such techniques for the estimation of drugs in biological samples is recommended. Other relatively economical and faster analytical techniques such as UV spectrophotometry [13–15] and potentiometry [16] have also been used for the determination of these drugs. The routine quality control analysis of pharmaceuticals demands for simple, economical and fast analytical techniques. The conductometric analysis might prove to be more suitable for the purpose as it is one of the most cost-effective techniques for drug analysis and offers simple, sensitive and rapid analytical procedures. The conductometric titration involves the measurement of conductance of solution, which varies due to interaction between ionic species before and after the end point.

Previously, we successfully utilized conductometric titration methods to perform the quantitative estimation of diphenhydramine hydrochloride [17] and pioglitazone hydrochloride [18] in a pure bulk form and pharmaceutical formulations. Moreover, this technique was also used by several other researchers for the determination of various pharmaceutical substances. However, to the best of our apprehension, no conductometric method was developed for quantification of tested drugs. Therefore, the present investigation was undertaken to develop a simple, economical and fast analytical method to quantitatively analyze four TK inhibitors imatinib, sorafenib, gefitinib and bosutinib in bulk drug and dosage forms using conductometry.

The functional moieties present in the selected drugs were methylpiperazine, pyridine and pyrimidine in imatinib; pyridinecarboxamide in sorafenib; morpholine and quinazoline in gefitinib and methylpiperazine, quinoline and carbonitrile in bosutinib (Figure 1), which get ionized in suitable solvents resulting in generation of negatively charged ions (bases) that are capable of binding to the Lewis acids (metal ions). Therefore, in the method development, principle of drug–metal ion interaction was applied, and in the titration vessel, the drug species was made to bind to metal cations, leading to alteration in the conductivity of the solution. Owing to the speed, simplicity and cost-effectiveness of the experimental procedure, the present method can be used for routine analysis of the selected drugs in quality control laboratories. Moreover, the procedure was eco-friendly as no organic solvents were used throughout the experiment.

2 Experimental procedure

2.1 Chemicals and reagents

The samples of imatinib, sorafenib, gefitinib and bosutinib were purchased from Sigma Aldrich (Steinheim, Germany). Copper sulphate (CuSO_4), ferrous sulphate (FeSO_4), ferric chloride (FeCl_3), silver chloride (AgCl) and sodium chloride (NaCl) were purchased from Merck, Germany. Starch, anhydrous lactose and magnesium stearate were acquired from Loba Chemie (India), and carboxymethyl cellulose CMC was obtained from Sigma Aldrich (Steinheim, Germany). The chemicals used in this experiment were of analytical grade, and double-distilled grade water was used to prepare the analytical solutions.

2.2 Instrumentation and conductometric titration

Conductometric titration was performed using portable conductivity/TDS meter (Jenway 470, Staffordshire, United Kingdom). Double-distilled grade water was produced in-house using Milli-Q (Millipore, Molsheim, France) water purifier system. An aliquot (10 mL) of metal ion solution was transferred to the titration vessel maintained at room temperature ($25 \pm 2^\circ\text{C}$), and the probe was immersed into the titration cell. It was followed by quantitative addition of sample solution containing one of the analyte drugs. After each 5 μL addition, the solution was stirred for 30 seconds, and the resulting conductance was recorded. Each observed conductance was used to calculate the corrected conductance values by following the reported procedure [19,20]. Considering conductivity as a linear function of dilution, Equation (1) is utilized for the calculation of correction of conductance [21].

$$\begin{aligned} \text{Conductance } (\Omega_{\text{correct}}^{-1}) \\ = \text{Conductance } (\Omega_{\text{obs}}^{-1}) \left[\frac{v_1 + v_2}{v_1} \right] \end{aligned} \quad (1)$$

where Ω_{obs}^{-1} and $\Omega_{\text{correct}}^{-1}$ are the observed and corrected electrolytic conductivity values, respectively, and v_1 and v_2 represent the initial and final volumes of each added titrant, respectively. A plot of corrected conductivities versus volumes of added drug solution (titrant) was prepared to determine the end point.

Figure 1: Chemical structure of imatinib (a), sorafenib (b), gefitinib (c) and bosutinib (d).

2.3 Preparation of 10 mM ammonium formate buffer (pH 3.5)

Accurately weighed quantity (63.06 mg) of ammonium formate was dissolved in 100 mL double-distilled water followed by adjustment of pH to 3.5 by adding dilute formic acid. The solution obtained was then filtered using 0.45 μ m filter.

2.4 Preparation of standard drug and metal ion solutions

Standard stock solution of imatinib (2 mM) was prepared by dissolving 59.0 mg of imatinib in 5 mL water. However, sorafenib, gefitinib and bosutinib stock solutions were prepared separately by dissolving 63.7 mg, 44.7 mg and 54.8 mg, respectively, into 5 mL freshly prepared ammonium formate buffer solution (pH 3.5) to achieve a concentration of 2 mM for each drug. Similarly, the standard stock solutions for metal ions were prepared by dissolving 11.6 mg, 32.4 mg, 30.4 mg, 49.8 mg and 28.6 mg of NaCl, FeCl₃, FeSO₄, CuSO₄ and AgCl, respectively, in 100 mL of double-distilled water to obtain a concentration of 2 mM for each solution. The stock solutions of drugs and metal ions were diluted appropriately with their respective diluents to achieve

working standards of required concentrations. All the prepared solutions were filtered using 0.45 μ m filter.

2.5 Preparation of pharmaceutical formulations

The pharmaceutical formulations for imatinib, sorafenib, gefitinib and bosutinib used in this study were prepared in-house and consisted of synthetic mixtures of each drug and placebo. To prepare the mixtures, 50 mg of each drug was added and mixed in calculated quantities of starch, anhydrous lactose, magnesium stearate and carboxymethyl cellulose.

2.6 Preparation of solutions from drug formulations

In-house mixtures equivalent to 59.0 mg, 63.7 mg, 44.7 mg and 54.8 mg of imatinib, sorafenib, gefitinib and bosutinib, respectively, were accurately weighed and transferred to separate 5 mL volumetric flasks. The mixture containing imatinib was dissolved in double-distilled water, while sorafenib, gefitinib and bosutinib were dissolved in ammonium acetate buffer (pH 3.5) to

obtain stock solutions of 2 mM concentration for each drug. The stock solutions were diluted appropriately with their respective diluents to attain working standards of required concentrations. All the prepared solutions were filtered using 0.45 μm filter.

2.7 Preparation of placebo solutions

The placebo was prepared by properly mixing the same amount of ingredients of pharmaceutical formulations without drugs. Equivalent amounts of placebo powder containing anhydrous lactose, starch, carboxymethyl cellulose and magnesium stearate were weighed and transferred to separate 5 mL volumetric flasks. The placebo mixture for imatinib was dissolved in double-distilled water, while sorafenib, gefitinib and bosutinib were dissolved in ammonium acetate buffer (pH 3.5). Further dilutions were made by following the procedure as described in the preparation of sample solutions. The final solutions were filtered through 0.45 μm Millipore filter.

2.8 Method validation

The final optimized conductometric method was validated according to the ICH and USP guidelines [22,23], and different validation parameters including specificity, sensitivity, linearity, precision, accuracy and solution stability were evaluated. This method was checked for specificity by conductometrically analyzing the placebo as well as blank solutions and comparing their conductivities with the ones observed from the analysis of sample solutions. The sensitivity of the method was examined through the calculation of limit of detection (LOD) and limit of quantification (LOQ) of all the analytes. Linearity of the developed conductometric method was evaluated at five concentration points including 1.0, 1.1, 1.2, 1.3 and 1.4 mM for each analyte separately. The calibration curve for each analyte was prepared by plotting corrected conductivities against the concentrations of the analyte. The regression analysis was performed by least square method, and y-intercept, slope and correlation co-efficient were calculated. For each concentration level, the six replicates were performed and conductance was measured.

For the determination of accuracy and precision of the present method, quality control solutions possessing

1.1, 1.2, and 1.4 mM concentrations at low (LQC), medium (MQC) and high levels (HQC) of analytes, respectively, were selected. These solutions were titrated on the same day at different times to evaluate intraday, whereas they were analyzed for three consecutive days to find out the interday precision and the accuracy of the proposed conductometric method. The precision was represented by calculating the %RSD of the conductance values, while the accuracy was determined by recovery studies at aforementioned three concentration levels. A %RSD of <2% and recovery in the range of 98–102% were considered as the acceptance criteria for method precision and accuracy, respectively. Each quality control solution was analyzed in six replicates. Recoveries of drug analytes in pharmaceutical formulations were tested using MQC (1.2 mM) sample at 80%, 100% and 120% concentrations. The standard addition method was employed for the preparation of recovery samples, which were analyzed in triplicates. The standard and test solutions were evaluated for stability after storing at laboratory temperature ($25 \pm 2^\circ\text{C}$) for 7 days. At the end of the storage time, the solutions were analyzed by using the current conductometric method in triplicate, and recoveries of all the analytes were calculated.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and discussion

3.1 Method optimization

According to Pearson's Hard-soft acid base theory (HSAB), depending on the mass, charge and polarizability, metals can behave as acids. The metals exhibiting low polarizability index are considered to be hard acids, whereas the ones with high polarizability indices are classified as soft acids, and the metals exhibiting intermediate properties are known to be borderline acids or bases. As per the HSAB theory, usually hard acids favorably coordinate with hard bases and the soft acids coordinate with soft bases [24]. Among the metal ions used in the present investigation, Na^+ and Fe^{3+} were hard acids, Ag^+ was soft acid and Cu^{2+} and Fe^{2+} were considered to be borderline acids. The drugs used in the present experiment possess ligands, which can complex with metal ions. These ligands are methylpiperazine, pyridine and pyrimidine in imatinib; pyridinecarboxamide in sorafenib; morpholine and quinazoline in gefitinib and

methylpiperazine, quinoline and carbonitrile in bosutinib [25,26].

The present investigation was a development and validation of a conductometric method for the quantitative estimation of four important anticancer agents, imatinib, sorafenib, gefitinib and bosutinib. The method was based on the principle of drug-metal ion interaction, where the ionizable moieties of the drug molecules complexed with certain metal ions. As a result of their complexation, the solution conductance was changed. The equivalence or end point of the titration was determined by the highest alteration in conductance values. The addition of analytical solution (titrant) to the metal ion solution present in the titration vessel led to the continuous decrease in the molar conductance of the solution until the end point. However, further addition of the drug solution resulted in a gradual increase in the conductance values. The reduction in molar conductance could be due to a decrease in metal ion concentration and the formation of lower mobility drug-metal ion complex, whereas the increase in molar conductance was due to an increase in free drug concentration in the titration cell. The curve between corrected conductance values and volume of drug solution (titrant) is shown in Figure 2.

During the optimization process, complexation behavior of certain metal ions including Ag^+ , Na^+ , Cu^{2+} , Fe^{3+} and Fe^{2+} with selected drugs was investigated. After remarkable efforts, Cu^{2+} was selected for the current analysis because Cu^{2+} exhibited superior binding interaction with all the selected drug molecules, and sharp end points were observed. Moreover, among the tested metal ions, better analytical results were obtained when CuSO_4 solution was used as a titrant. The medium to dissolve analytes in conductometric titration may affect the end point as well as the analytical results. In this experiment, to find out the suitable medium, water was tried first, because water is considered to be the most appropriate and economical medium for conductometric determinations. However, only imatinib showed optimum solubility in water and displayed sharp end point with acceptable analytical performance, whereas for sorafenib, gefitinib and bosutinib, water could not be used as medium because of poor solubility, resulting in undistinguishable end point and poor analytical results. Consequently, different acidic and basic buffers were examined, and finally, the most suitable medium to dissolve sorafenib, gefitinib and bosutinib was identified to be ammonium formate buffer (pH 3.5) as all the analytes exhibited

Figure 2: Conductometric titration curve showing corrected conductance values against the volume of imatinib (a), sorafenib (b), gefitinib (c) and bosutinib (d) solutions added in Cu^{2+} solution (10 mL) in the titration vessel.

optimum solubilities and satisfactory analytical results were achieved in this buffer. Subsequently, metal ions were dissolved in water in the conductometric analysis of imatinib and buffer for the determination of sorafenib, gefitinib and bosutinib.

It is well known that temperature has a marked influence on the analytical results, and conductance of the solutions can be changed with temperature in conductometry. Therefore, after finding out the suitable solution medium, the influence of temperature on the conductivities of the tested drugs was also studied. A temperature range between 25 and 45°C was tested. However, during trials, no significant alterations in the solution conductance values were observed with an increase in the temperature. Consequently, laboratory temperature ($25 \pm 2^\circ\text{C}$) was finalized for the current analysis. To determine the optimum concentrations of drug analytes and metal ion, which can offer stable conductivities and sharp equivalence point, several concentrations were tried during optimization. The concentrations of drugs as well as metal ion less than 0.5 mM were found to be not optimum for the current conductometric determination as poor inflection at equivalence point and unstable conductance values were observed

at these concentrations. After several trials, it was found that the concentration between 1.0 and 1.5 mM for analyte drugs as well as metal ions displayed acceptable analytical results and sharp end points. Furthermore, in this investigation, best results were obtained when equimolar concentrations for drug and metal ion solutions were used.

3.2 Method validation

The optimized method for estimating imatinib, sorafenib, gefitinib and bosutinib by the conductometric analysis was evaluated for various validation parameters in accordance with the ICH/USP guidelines. To establish the linearity of the optimized method, analytical solutions possessing a range of concentrations of imatinib, sorafenib, gefitinib and bosutinib were conductometrically analyzed. A calibration curve was constructed for each drug by plotting corrected conductance values versus concentrations of analyte (Figure 3). Consequently, linear plots were obtained for all four analytes over a concentration range of 1.0–1.4 mM, and $R^2 > 0.99$

Figure 3: Calibration graph for imatinib (a), sorafenib (b), gefitinib (c) and bosutinib (d) displaying good linearity of the proposed method in the analyte concentration range of 1.0–1.4 mM, $R^2 > 0.99$.

Table 1: The linearity data of the proposed conductometric method

Parameter	Imatinib	Sorafenib	Gefitinib	Bosutinib
Y-intercept	23.56	27.64	-213.92	-7.94
Slope	13 333	7731.4	15 397	1023.4
Standard error (intercept)	124.44	107.61	194.66	42.058
Standard error (slope)	102.99	89.058	161.10	34.808
Correlation coefficient (R^2)	0.9998	0.9996	0.9997	0.9965
Standard deviation of residuals from line ($Sy \cdot x$)	32.568	28.162	50.945	11.007

was achieved, indicating good linearity of the proposed method. The linearity data have been summarized in Table 1.

The limit of detection (LOD) values for imatinib, sorafenib, gefitinib and bosutinib were calculated to be 0.00021, 0.00037, 0.00037 and 0.0028 mM, whereas the limit of quantification (LOQ) values were found to be 0.00071, 0.00122, 0.00122 and 0.0093 mM, respectively. Remarkably lower LODs and LOQs for all the drugs indicated that the developed conductometric method was highly sensitive. The method specificity was demonstrated via comparison of the conductometric plot drawn from all the drug solutions, with those obtained from analyzing of blank and respective placebo. After careful observation, no sharp change was seen in the conductance during blank and placebo titrations, and there was no significant inflection at the end points. This has indicated that there was no interference from the solution medium, and the components of the placebo in the conductometric analysis using the proposed method and hence, the method was considered to be specific for the selected analytes.

The precision and the accuracy of the proposed conductometric method were evaluated by analyzing the quality control samples for all the drug analytes at low,

intermediate and high concentrations in triplicate. The % RSD of the corrected conductance recorded for each drug in both intraday and interday analyses was less than 2%, which was considered to be in the acceptable limit. The accuracy of the method was determined using the same solutions by calculating the recovery of respective drugs at all concentration levels. The recovery values of all the drugs in intraday and interday analysis were within the $100 \pm 2\%$ range. The obtained results suggested that the current method was precise and accurate. The precision and the accuracy data are presented in Table 2. After performing the precision and the accuracy experiment, the medium quality control (MQC) solutions was kept aside for 7 days at laboratory temperature ($25 \pm 2^\circ\text{C}$) to investigate the stability of imatinib, sorafenib, gefitinib and bosutinib in their respective solutions. The recovery of all the analytes after end of the storage time was calculated and found to be within the $100 \pm 2\%$ range. The results showed no significant loss of analytes in any of the four drug solutions, and all the analytes were stable in their analytical solutions, which can be used for at least 7 days at laboratory temperature without degradation. The results of the solution stability are presented in Table 3.

Table 2: Results of precision and accuracy of the developed conductometric method for imatinib, sorafenib, gefitinib and bosutinib analyzed at low, intermediate and high concentration levels

	Analyte concentration (mM)	Imatinib	Sorafenib	Gefitinib	Bosutinib
Intraday precision and accuracy ^a					
% RSD of corrected conductance (mean% recovery)	LQC ^b (1.1 mM)	0.035 (99.91)	0.260 (100.53)	0.081 (99.84)	1.599 (101.56)
	MQC ^c (1.2 mM)	0.023 (100.43)	0.078 (100.45)	0.089 (100.01)	0.569 (100.40)
	HQC ^d (1.4 mM)	0.019 (100.05)	0.093 (100.17)	0.100 (99.48)	0.743 (101.13)
Interday precision and accuracy ^a					
% RSD of corrected conductance (mean% recovery)	LQC (1.1 mM)	0.040 (99.92)	0.040 (99.92)	0.184 (100.51)	0.114 (99.85)
	MQC (1.2 mM)	0.046 (100.42)	0.046 (100.42)	0.121 (100.57)	0.124 (100.04)
	HQC (1.4 mM)	0.053 (100.04)	0.053 (100.04)	0.097 (100.22)	0.152 (99.46)

^a $n = 6$. ^b LQC. ^c MQC. ^d HQC represents low, medium and high concentration of quality control solutions.

Table 3: Solution stability data of imatinib, sorafenib, gefitinib and bosutinib analyzed by the proposed conductometric method using medium quality control solutions

Drugs	Storage conditions	Recovery (percent RSD) ^a
Imatinib	25 ± 2°C, 7 days	100.26 (0.145)
Sorafenib	25 ± 2°C, 7 days	100.68 (0.246)
Gefitinib	25 ± 2°C, 7 days	99.96 (0.512)
Bosutinib	25 ± 2°C, 7 days	100.56 (0.456)

^a $n = 6$.

3.3 Application to determine imatinib, sorafenib, gefitinib and bosutinib in their respective dosage forms

After validation, the present conductometric method was evaluated for its applicability for the estimation of imatinib, sorafenib, gefitinib and bosutinib in their respective dosage forms, which were prepared in-house. The tested drugs were mixed separately with measured quantities of anhydrous lactose, carboxymethyl cellulose, magnesium stearate and starch. These dosage forms were then quantitatively analyzed using the developed method. First, the conductometric titration curve for each drug solution was carefully examined and compared with those obtained from titration of placebo solution. The method showed good specificity towards the analyses of all drug formulations because no interferences in the conductance values were recorded

from placebo solution, and no sharp inflection of conductance values near the end point was observed. Furthermore, the accuracy of the method was also established by performing the recovery experiment at 80%, 100% and 120% of the target MQC concentration (1.2 mM) using drug formulations, and the results for all tested analytes were within the acceptable limit (100 ± 2%). The acceptable recovery of analytes from their respective dosage forms suggested suitability of the current method for quantitative analyses of the tested drugs in their respective dosage forms. The observed recovery of imatinib, sorafenib, gefitinib and bosutinib in their formulations is presented in Table 4.

4 Conclusion

In this study, complex forming behavior of important anticancer agents imatinib, sorafenib, gefitinib and bosutinib with metal ions was employed to develop a new conductometric method for quantitation in bulk and dosage forms. The analytical procedure involved gradual addition of drug solution to the Cu²⁺ solution in the conductometric titration vessel, resulting in a sharp change in the conductance values of the solution owing to the interaction between the test drugs with Cu²⁺. The point of maximum conductance variation was considered as end point. The method validation results indicated that the proposed method was accurate,

Table 4: Recovery results of imatinib, sorafenib, gefitinib and bosutinib in their dosage forms prepared in-house

Drugs	Recovery solution concentrations (mM)	Targeted concentrations (%)	Amounts of drugs recovered (mM)	Recovery ± %RSD ^a
Imatinib	0.96	80	0.962	100.20 ± 0.562
	1.2	100	1.205	100.42 ± 0.038
	1.44	120	1.438	99.86 ± 0.821
Sorefenib	0.96	80	0.963	100.31 ± 0.784
	1.2	100	1.209	100.75 ± 0.159
	1.44	120	1.438	99.86 ± 0.547
Gefitinib	0.96	80	0.957	99.69 ± 1.106
	1.2	100	1.200	100.00 ± 0.100
	1.44	120	1.439	99.93 ± 1.162
Bosutinib	0.96	80	0.963	100.31 ± 1.184
	1.2	100	1.221	101.75 ± 0.954
	1.44	120	1.438	99.86 ± 1.112

^a $n = 3$.

precise, sensitive and linear. The proposed method was applied for the assay of imatinib, sorafenib, gefitinib and bosutinib in their respective pharmaceutical dosage forms, which was formulated in-house by mixing each drug with placebo components. No interferences were observed from blank and placebo solutions, indicating good specificity of the method for all drugs in the formulation analysis. The fast analysis and cost-effectiveness are considered to be the major advantages of the method in addition to having acceptable analytical performance and being environmental friendly. As a result, this method can be used for the routine analysis of these drugs in quality control laboratories.

Acknowledgments: This project was funded by Deanship of Scientific Research, Jazan University, Jazan, Saudi Arabia, under 6th Future Scientist Project program (Grant number: FR6-51).

Conflict of interest: The authors declare no conflict of interest.

References

- [1] Roskoski R. A historical overview of protein kinases and their targeted small molecule inhibitors. *Pharmacol Res.* 2015;100:1–23.
- [2] Cohen P. Protein kinases – the major drug targets of the twenty-first century? *Nat Rev Drug Discov.* 2002;1:309–15.
- [3] Krause DS, van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med.* 2005;353:172–87.
- [4] Druker BJ. Imatinib mesylate in the treatment of chronic myeloid leukaemia. *Expert Opin Pharm.* 2003;4:963–71.
- [5] Gergely PA, Murnyák B, Bencze J, Kurucz A, Varjas T, Gombos K, et al. Tyrosine kinase inhibitor imatinib mesylate alters DMBA-induced early onco/suppressor gene expression with tissue-specificity in mice. *BioMed Res Int.* 2019;8670398, doi: 10.1155/2019/8670398
- [6] Jackson KD, Durandis R, Vergne MJ. Role of cytochrome P450 enzymes in the metabolic activation of tyrosine kinase inhibitors. *Int J Mol Sci.* 2018;19:2367–82.
- [7] Syed YY, McCormack PL, Plosker GL. Bosutinib: a review of its use in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. *BioDrugs* 2014;28:107–20.
- [8] Alhazmi HA, Moraya DA, Alahdal E, Kariri M, Al Bratty M, Rehman Z, et al. Ultrafast monolithic HPLC method for simultaneous quantification of the anticancer agents, imatinib and sorafenib: Application to tablet dosage forms. *Trop J Pharm Res.* 2018;17:1127–34.
- [9] Demir GM, Değim IT. Determination of imatinib mesylate and erlotinib hydrochloride in mice plasma using ultrahigh performance liquid chromatography method, *Kuwait J Sci.* 2018;45:57–63.
- [10] Merienne C, Rousset M, Ducint D, Castaing N, Titier K, Molimard M, et al. High throughput routine determination of 17 tyrosine kinase inhibitors by LC–MS/MS. *J Pharm Biomed Anal.* 2018;150:112–20.
- [11] Gotze L, Hegele A, Metzelder SK, Renz H, Nockher WA. Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma. *Clin Chim Acta.* 2012;413:143–9.
- [12] Lankheet NA, Hillebrand MJ, Rosing H, Schellens JH, Beijnen JH, Huitema AD. Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry. *Biomed Chromatogr.* 2013;27(4):466–76.
- [13] Bende G, Kollipara S, Sekar V, Saha R. UV-spectrophotometric determination of imatinib mesylate and its application in solubility studies. *Pharmazie.* 2008;63(9):641–5.
- [14] Ravisankar P, Babu PS, Taslim SM, Kamakshi K, Manasa RL. Development and validation of UV-spectrophotometric method for determination of sorafenib in pharmaceutical dosage form and its degradation behaviour under various stress conditions. *Int J Pharm Sci Rev Res.* 2019;56:12–7.
- [15] Chakravarthy VA, Sailaja BBV. 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. *Eur J Pharm Med Res.* 2016;3: 609–24.
- [16] Karim SEA, El-Nashar RM, Abadi AH. Potentiometric determination of imatinib under batch and flow injection analysis conditions. *Int J Electrochem Sci.* 2012;7:9668–81.
- [17] Al-Bratty M, Hashem H, Noureldeen A, Manoharan G, Towhar F. Conductometric determination of the antihistaminic diphenhydramine hydrochloride using silver nitrate as a titrant. *Int J Pharm Sci.* 2015;7(3):72–6.
- [18] Al-Bratty M, Hashem H, Eranhiyil S. Visual, potentiometric and conductometric acid-base titrations for determination of antidiabetic pioglitazone hydrochloride. *Int J Biol Pharm Allied Sci.* 2016;5:93–103.
- [19] Vogel AJ. Textbook of quantitative inorganic analysis. 4th ed. London: Longman; 1978. pp. 367.
- [20] Lingane JJ. Electroanalytical Chemistry. 2nd ed. New York: Interscience; 1958. p. 90
- [21] Ayad MA, Abdellatif HE, Hosny MM, Sharaf YA. Conductometric titration method for determination of naftidrofuryl oxalate, propafenone HCl and sotalol HCl using silver nitrate. *Eur J Chem.* 2012;3:332–6.
- [22] International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology, Q2 (R1); November, 2005. Available online: http://www.ipqpubs.com/wp-content/uploads/2011/09/Q2_R1_Guideline.pdf (accessed on 03 September 2019)
- [23] Center for Drug Evaluation and Research, US Food and Drug Administration. Reviewer Guidance, Validation of Chromatographic Methods; FDA, Rockville, MD; 1994. Available online: <https://www.fda.gov/downloads/Drugs/Guidances/UCM134409.pdf> (accessed on 03 September 2019)
- [24] Lopachin RM, Gavin T, Decaprio A, Barber DS. Application of the Hard and Soft, Acids and Bases (HSAB) theory to toxicant–target interactions. *Chem Res Toxicol.* 2012;25:239–51.

- [25] Rahimi-Nasrabadi M, Ganjali MR, Gholivand MB, Ahmadi F, Norouzi P, Salavati-Niasari M. A cyclic voltammetry investigation of the complex formation between Cu^{2+} and some Schiff bases in binary acetonitrile/dimethylformamide mixtures. *J Mol Struct.* 2008;885:76–81.
- [26] Rahimi-Nasrabadi M, Ahmadi F, Pourmortazavi SM, Ganjali MR, Alizadeh K. Conductometric study of complex formations between some substituted pyrimidines and some metal ions in acetonitrile and the determination of thermodynamic parameters. *J Mol Liq.* 2009;144:97–101.