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Original Research Article

Ultrafast monolithic HPLC method for simultaneous quantification of the anticancer agents, imatinib and sorafenib: Application to tablet dosage forms

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Abstract

Purpose: *To develop and validate a simple ultrafast monolithic high performance liquid chromatography (HPLC) method for the simultaneous quantification of two anti-cancer agents, imatinib and sorafenib, in pure form and tablet preparations.*

Method: *Chromatographic separation was accomplished using Chromolith flash RP-18 HPLC-column (25 - 4.6 mm; macropores, 2 µm; mesopores, 13 – 15 nm). The optimum mobile phase composition of ammonium acetate buffer (10 mM, pH 8.5) and methanol at ratio of 35:65 v/v was used. Effluent flow rate was adjusted to 1.0 mL/min and the analysis was performed at 250 nm wavelength. The developed method was evaluated for specificity, linearity, precision and accuracy.*

Results: *The method offered a linear relationship over the concentration range of 1 - 16 µg/ml (correction coefficient, R^2 = 0.9999) for both analytes. Limit of detection (LOD) was 0.1891 and 0.1888 µg/ml while limit of quantification (LOQ) was 0.6303 and 0.6294 µg/ml for imatinib and sorafenib, respectively. Mean recovery was within 100 ± 2 %. The utility of the new method was demonstrated by its successful use for the analysis of commercially available tablet formulations of both drugs.*

Conclusion: *The developed method is fast and economical, and is being recommended for routine analysis of imatinib and sorafenib in bulk drug and tablet dosage forms in quality control laboratories.*

Keywords: *RP-HPLC, Chromolith, Imatinib, Sorafenib, Validation, Quality control*

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INTRODUCTION

The development of tyrosine-kinase inhibitors as chemotherapeutic agents had remarkable impact on the management of a variety of malignancies. The tyrosine-kinase has a major function in the signals transduction for cellular growth [1]. Imatinib (IMB, Figure 1a), chemically a 2-phenylaminopyrimidine derivative, is a chemotherapeutic agent, used as first line drug to treat chronic myeloid leukemia, also used for gastrointestinal stromal tumors, myelodysplastic syndrome and other cancer types [2,3]. Imatinib acts by selectively inhibiting the protein tyrosine-kinase in a competitive manner. It interacts to the

ATP-binding sites of BCR-Abl type tyrosine-kinase present in the cancer cells, blocking the phosphorylation of the substrate, therefore, stopping the cell proliferation and hence cell growth [4].

Sorafenib (SFB, Figure 1b), a diarylurea derivative containing pyridine-2-carboxamide moiety, is a multi-kinase inhibitor used in colorectal cancer and hepatocellular carcinoma [5]. As monotherapy or in combination with other antitumor agents, sorafenib has displayed significant anticancer activity against different tumor types. In various preclinical and clinical studies, sorafenib has been proved to hinder cell proliferation, reduce cancer growth and angiogenesis, in addition to induction of apoptosis in tumor cells [6-8]. Initially, it has been developed as Raf1-kinase pathway inhibitor, however, it also binds with receptor tyrosine-kinase such as FLt-3, c-KIT, VEGFR-2 and VEGFR-3 [9, 10]. The clinical studies on imatinib, sorafenib and nilotinib have shown an increased therapeutic potential, especially on castrate-resistant prostate cancer and suggested that combination of two or three tyrosine-kinase inhibitors are safe and efficacious [11, 12].

An in-depth literature survey evidenced that many analytical techniques are developed for the estimation of IMB in single by UV-spectrophotometry [13,14], potentiometry [15], HPLC [16-18] and HPTLC [19] and with other drugs by HPLC and LC-MS [20-22], whereas SFB in single and combined dosage forms were estimated by UV-spectrophotometry [23,24] and different chromatographic methods such as HPLC [25,26] and HPTLC [27]. However, to the best of our knowledge, no reverse-phase HPLC has been reported for simultaneous analysis of IMB and SFB in tablet preparations. The aim of the current study was to develop a rapid and economical monolithic HPLC method for estimation of IMB and SFB, validate as per ICH method validation guidelines [28] and finally to apply for the determination of IMB and SFB in tablet dosage form.

EXPERIMENTAL

Materials

The reference standards of IMB and SFB, ammonium acetate, ammonium hydroxide, acetonitrile and methanol (HPLC grade) were purchased from Sigma Aldrich, Germany. The tablet dosage forms of IMB and SFB were obtained from Prince Mohammed Bin Nasser Hospital, Jazan, Saudi Arabia.

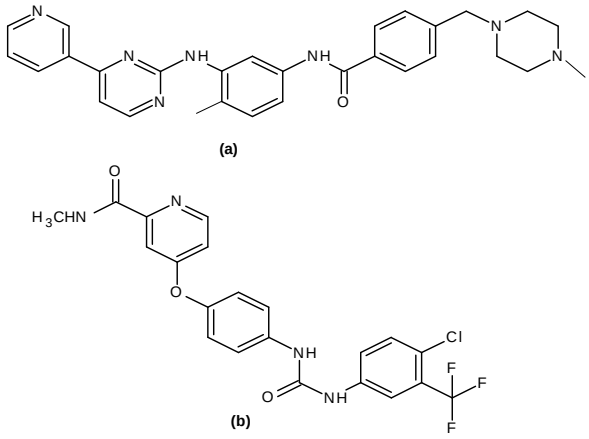

Figure 1: Chemical structures of (a) sorafenib and (b) imatinib

Instrumentation and chromatographic conditions

The present liquid chromatography experiment was performed on HPLC system (Waters Breeze 1525 HPLC system, Netherland) equipped with UV- visible detector (Waters 2489) and auto sampler (Waters 2707). Chromolith Flash RP-C18 column 25 x 4.6 mm, macropores 2 µm, mesopores (skeleton) 13 - 15 nm, maintained at ambient temperature (25 °C) was used for chromatographic separation and mobile phase containing ammonium acetate buffer (10 mM, pH 8.5) and methanol (35:65 v/v) was pumped at1.0 mL/min flow rate. The injection volume of 20 µL was used and the detector was set at a wavelength of 250 nm. Analytical balance (Kern & Sohn GmbH, Germany) was utilized throughout the study. HPLC grade water used in this work was produced by Millipore water purification system (Millipore, USA) in our laboratory.

Preparation of ammonium acetate buffer (10 mM, pH 8.5)

Ammonium acetate (77.1 g) was dissolved in 1000 ml ultrapure water. The pH was adjusted to 8.5 using 35 % ammonium hydroxide solution. Then the buffer was filtered through 0.45 µ nylon membrane filter.

Preparation of mobile phase

HPLC grade methanol was added to the acetate buffer (pH 8.5) as an organic modifier at ratio of 35:65 v/v, mixed and sonicated to degas it.

Preparation of standard stock solutions

The stock standard solutions of IMB and SFB were prepared by transferring accurately

weighed amount (10 mg) of each drug separately into 10 mL volumetric flasks, dissolved in a mixture of methanol and acetonitrile (3:2) and volumes were adjusted with the same mixture to achieve a final concentration of 1.0 mg/mL.

Preparation of working standard solutions

Aliquots from standard stock solutions of IMB and SFB were diluted with a mixture of methanol and water (1:1) to obtain working standard solution containing a mixture of both analytes at concentration of 8 µg/mL.

Sample preparation

The average weight of IMB and SFB tablets were determined by weighing 20 tablets, finely crushed and the powder equivalent to 10 mg of IMB and SFB were taken separately into 10 mL volumetric flasks. To each flask, 5 mL of methanol was added and sonicated for 20 min, the volumes were adjusted with a mixture of methanol-water (1:1) to achieve sample stock solutions (1.0 mg/mL) for both the drugs. The solutions were filtered through nylon filter (0.45 µ). 0.4 mL of each filtrate was placed in a 50 mL volumetric flask and made up to volume with mobile phase to achieve a target concentration 8 µg/mL for both drugs.

Method validation

The developed ultrafast monolithic liquid chromatographic method was validated in compliance with ICH guidelines of Validation of Analytical Procedures [28]. The following parameters were evaluated:

System suitability

To determine the system suitability, working standard solution was injected in the system and analyzed using previously described chromatographic conditions. The system suitability parameters such as peak resolution, theoretical plate count, tailing factor and the % RSD of area of six replicate injections for both analytes were recorded.

Linearity

To demonstrate the linearity of the present HPLC method, a series of portions from stock standard solutions of IMB and SFB were diluted to obtain five sets of working standard solutions of concentration range 1 – 16 µg/mL containing mixture both analytes. The solutions were analyzed in six replicates, the peak area of IMB and SFB were recorded and calibration graphs were plotted using peak area of each component against the corresponding concentrations. The regression equation was obtained.

Precision and accuracy

The working standard solutions at low (2 µg/mL, LQC), medium (8 µg/mL, MQC) and high (16 µg/mL, HQC) quality control levels, which are within the linearity range were analyzed. Intra-day precision and accuracy experiment was carried out by analyzing the above solutions at three different times on the same day, while the inter-day precision and accuracy was assessed by screening the solutions over three consecutive days. The precision results were expressed as % RSD of the peak area and the accuracy of the method was presented as % recovery of IMB and SFB at all three concentration levels.

Limit of detection (LOD) and limit of quantification (LOQ)

The LODs and LOQs of IMB and SFB were estimated using the equation LOD = 3.3 × SD/s; and LOQ = 10 × SD/s, where, 'SD' is standard deviation of y-intercept of the regression line and 's' is slope of calibration graph.

Solution stability

To evaluate the stability, sample and standard solutions were stored at room temperature (25 °C) for 12 h (bench-top stability), in refrigerator (4 °C for 14 days and -20 °C for 30 days). The solutions were taken out at the end of each storage time and analyzed by the proposed method. The results obtained were compared with the freshly prepared solution.

RESULTS

In the present study, a simple, fast and economical HPLC method for simultaneous determination of imatinib and sorafenib has been developed. The optimum chromatographic conditions for good separation between the two analytes was achieved on isocratic elution mode with mobile phase composed of 10 mM ammonium acetate buffer (pH 8.5) and methanol (35:65 v/v). The mobile phase flow rate was maintained at 1.0 mL/min, pumping through Chromolith Flash RP- C18 HPLC column, 25 - 4.6 mm, macropores 2 µm, mesopores (skeleton) 13 – 15 nm, set at ambient temperature (≈ 25 °C). The data was monitored at 250 nm. IMB and SFB were observed at 1.2 and 3.8 min and a runtime of 5 min was fixed throughout the analysis. The representative

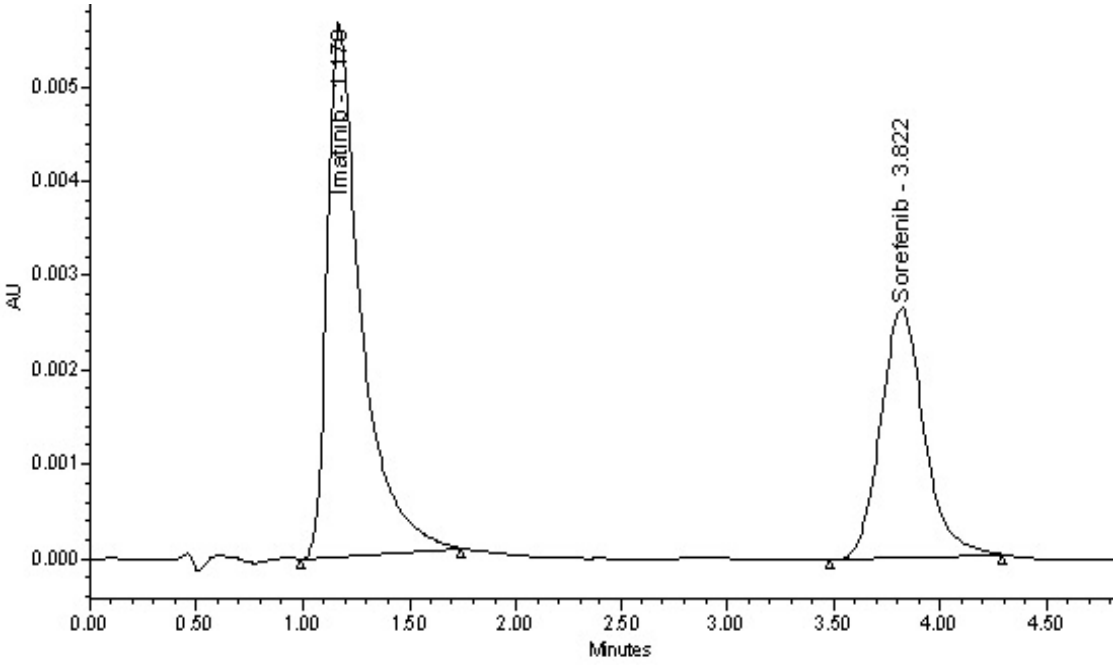

Figure 2: Representative chromatogram showing well separated peaks of IMB and SFB

chromatogram showing good separation and symmetric peak shape of IMB and SFB has been depicted in Figure 2. The method was evaluated in compliance with ICH guidelines, in terms of system suitability, linearity, precision and accuracy, specificity and solution stability. The retention times of both the analytes were constant during the course of analysis. The system suitability parameters such as USP tailing, USP resolution and USP plate count for IMB and SFB were observed to be in the acceptable range set by CDER, US FDA [29]. The peaks were in good shape and symmetrical and the % RSD of six replicate injections was recorded to be < 2 % for both analytes. The observed system suitability parameters have been summarized in Table 1.

Table 1: System suitability parameters observed for imatinib and sorafenib in this study

System suitability parameter	Imatinib	Sorafenib
Retention time (min)	1.2	3.7
USP tailing factor	1.72	1.25
USP resolution		7.8
USP plate count	2354	3876
%RSD of peak area (n = 6)	0.49	0.63

The linearity of the method was established at five concentration levels (1 – 16 µg/mL), the calibration graph was made by plotting the peak area against concentrations of IMB and SFB. Excellent linearity was observed as the correlation coefficients of R^2 = 0.9999 was obtained from the calibration plots of both IMB and SFB (Figure 3). Intra-day and inter-day analysis of the three sample quality controls (LQC, MQC and HQC) by the developed method showed good precision, the % RSD for six repeated runs of each quality control sample was recorded to be < 2%. Furthermore, the method has offered very accurate results as the recovery of IMB and SFB in intra- and inter-day analysis were within in 100 ± 2 % for the three sample quality controls. The precision and accuracy data have been represented in Table 2. The applicability of the developed method has been demonstrated by analysis of the tablet formulation of both the drugs. The recovery study was performed at 50, 100 and 125 % levels of the target concentration (8 µg/mL). The recovery data of tablet formulations has been summarized in Table 3.

The LODs and LOQs for the proposed method were 0.1891 and 0.6303 µg/mL for IMB and 0.1888 and 0.6294 µg/mL for SFB, respectively. The stability of the analytical solutions (8 µg/mL) was tested by storing at different conditions as mentioned in the experimental section. The % RSD of the peak area of six replicate injections was calculated to be less than 2 % and the percent recovery for both IMB and SFB were observed within 100 ± 2 % (Table 4).

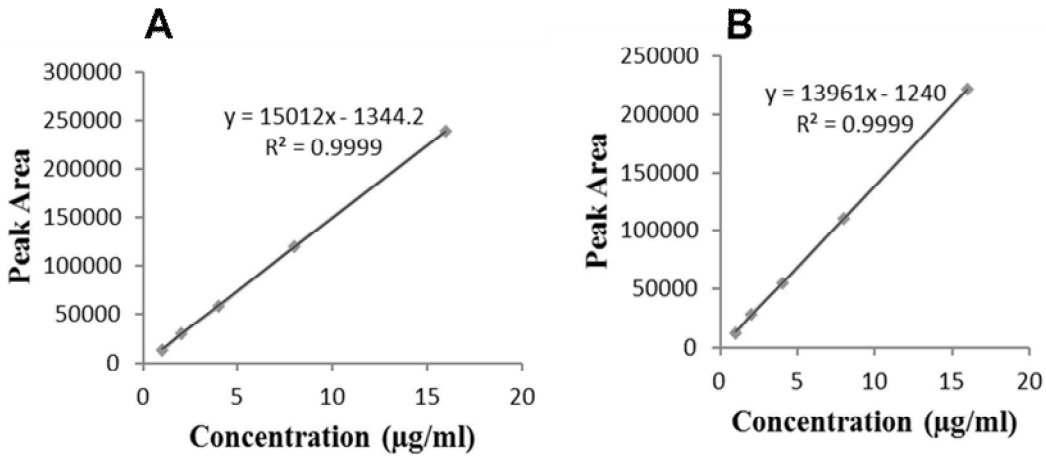

Figure 3: Calibration curve for A: imatinib and B: sorafenib

Table 2: Intra-day and inter-day analysis results of the developed method at three sample quality control levels for imatinib and sorafenib

Parameter	Sample concentration	%RSD of peak area (% recovery)	
		Imatinib	***Sorafenib***
Intra-day	LQC (2µg/mL)	1.099 (101.41)	1.541 (102.01)
	MQC (8µg/mL)	0.987 (100.96)	0.898 (99.92)
	HQC (16µg/mL)	1.863 (100.41)	1.298 (100.32)
Inter-day	LQC (2µg/mL)	1.402 (100.19)	1.521 (100.96)
	MQC (8µg/mL)	1.114 (100.61)	1.031 (99.68)
	HQC (16µg/mL)	1.377 (100.24)	1.354 (101.86)

Key:*n = 6; LQC, MQC and HQC are low, medium and high quality control concentration levels respectively*

Table 3: Recovery data for imatinib and sorafenib in tablet dosage forms

Analyte	Concentration level	Recovery (%)	%RSD
Imatinib	50%	101.4	0.92
	100%	100.8	0.47
	125%	98.9	0.73
Sorafenib	50%	100.4	0.90
	100%	100.5	0.34
	125%	99.4	0.34

**n = 6*

DISCUSSION

At the beginning, chemical characteristics and hence retention behavior of IMB and SFB was studied to find out the optimum chromatographic separation between two components. It is well known that the background noise of chromatogram is generally more at UV wave-length ≤ 220. Therefore, the UV absorption intensity for IMB and SFB was tested at wavelength 220 – 300 nm. Finally a wavelength of 250 nm was found to show good absorption for both analytes, consequently, an excellent baseline with negligible noise was obtained.

Table 4: Solution stability data at different storage conditions

Storage condition	Duration	%Recovery (%RSD)
Imatinib		
Room temperature (25°C)	12 h	99.86 (1.44)
Refrigerator temperature (4°C)	14 days	100.12 (1.51)
Freezer temperature (-20°C)	30 days	100.46 (0.62)
Sorafenib		
Room temperature (25°C)	12 h	100.21(2.1)
Refrigerator temperature (4°C)	14 days	101.96 (1.60)
Freezer temperature (-20°C)	30 days	101.15 (0.25)

**Solutions were injected in six replicates and the analysis was carried out using solution of 8 µg/ml concentration.*

Different mobile phase compositions with acidic and basic buffers were tried to achieve fast and good separation. The mobile phase composed of ammonium acetate buffer and methanol / acetonitrile at various proportions were tried. With acidic buffer, no proper separation was observed between IMB and SFB. Various trials have also been taken using mobile phase containing basic acetate buffer (pH 8.5) and methanol at different proportions. With a mobile phase ratio of 30:70 v/v of acetate buffer pH 8.5 and methanol, good separation between IMB and SFB with symmetric peak shape was achieved, however, the first peak (IMB) was very close to

the front peak, hence this composition was not considered as final (Figure 3). Subsequently, the mobile phase optimization was done by minor change in composition and finally the peak due to IMB (first peak) has been separated from the front peak and the optimum mobile phase consisting of ammonium acetate buffer (pH 8.5) and methanol (35 : 65 v/v) was finalized for this analysis.

The use of mobile phase containing organic volatile salts such as ammonium acetate and volatile organic solvent such as methanol makes this method compatible with mass spectrometry [30] which allows easy method transfer to LC/MS. Furthermore, the use of cheap organic solvent (methanol) makes this method more economical and useful for routine analysis in quality control laboratories. The mesoporous skeleton as in Chromolith columns enhance the permeability of the column and prevents the generation of high back pressure [31,32] which probably is helpful to extend the life of HPLC - pump and other instrumental components. The newly developed HPLC method offers a quick separation with reduced back pressure within a 5 min runtime.

The system suitability was determined using the chromatograms of standard solution for both analytes at the beginning and observed throughout the analysis to ensure the performance of the HPLC system. The system suitability parameters such peak tailing, resolution, theoretical plate count and the %RSD of six replicate injections were with the acceptance limits, indicating the suitability of the HPLC - system to perform the experiment.

A linear relationship between peak area of IMB and SFB and the concentrations in the range of 1 - 16µg/mL was recorded from the calibration plots (correlation coefficients, R^2 = 0.9999 for both analytes), which suggested that the proposed method is linear. Three sample quality controls were prepared within the calibration curve range at low (LQC, 2 µg/mL), medium (MQC, 8 µg/mL) and high (HQC, 16 µg/mL) quality control levels and used to assess the precision and accuracy of the developed method. The intra-day and inter-day precision and accuracy data were within the acceptable range and found to meet the criteria of the ICH guidelines. These results indicated the developed chromatographic method is precise and accurate and hence, can be used for the quantitative estimation of imatinib and sorafenib. The relatively lower LOD and LOQ values indicate the sensitivity of the method. The chromatograms of sample and standard solutions were compared with that obtained from blank for the evaluation of the specificity of the proposed method. No interference was observed at the retention time of both the analytes, which suggested that the developed method is specific for the analysis of IMB and SFB.

The solution stability was evaluated by storing at different conditions including, room temperature (bench-top stability) for 12 h, the average time for analysis; refrigerator for medium period (14 days) and in freezer (-20°) for long term stability (30 days). The stability data has suggested that the solutions were stable at all the tested conditions and the solutions can be stored and used again without any significant loss.

The developed HPLC method was successfully utilized for quantification of IMB and SFB in tablet preparations available in the local community pharmacies. The observed system suitability parameters were within the acceptable range and no interference from tablet excipients was observed at the retention times of both the analytes. The recovery of imatinib and sorafenib in tablet formulations were assessed by standard addition method. The recovery samples were prepared by spiking standard to the tablet sample solution (50, 100 and 125 % of the target concentration of 8 µg/ml for both analytes). The quantities of IMB and SFB recovered were in good agreement with the label claims of the respective tablet formulations. The attained recoveries were found to be in range of 100 % ± 2 for both drug components. Consequently, the developed method is recommended for the quality control analysis of IMB and SFB in tablet formulations at the pharmaceutical production stages.

CONCLUSION

An ultrafast HPLC method has been successfully developed for the simultaneous determination of imatinib and sorafenib. Both anlaytes are well separated within short runtime. The method has utilizes a monolithic chromolith column which offers fast separation of analytes, in addition to low back pressure. The developed method is fast, specific, precise and accurate, and has very good linearity and recovery for both analytes. Further, the applicability of the method was proved by good recovery in the tablet samples. An important feature of the method is that it may be useful for bioanalysis and pharmacokinetic studies of imatinib and sorafenib. The method is also suitable bulk and solid dosage formulations of the two anticancer drugs.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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