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Professional paper

Development of Green RP-HPLC method for determination of sorafenib in film-coated tablets

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Abstract

A new green RP-HPLC method for the determination of sorafenib in film-coated tablets was developed and validated. The chromatographic separation was carried on a LichroCART® 60 RP-select B column, using isocratic elution mode, with a mobile phase consisting of a mixture of phosphate buffer, pH 6.0 and ethanol (45:55% *V/V*), maintaining the column temperature at 30 ºC. The detection wavelength was set at 265 nm. The method was validated according to the ICH guideline, with the focus on specificity, linearity, sensitivity, accuracy, precision, and robustness. The results for the greenness assessment, obtained with the Analytical Method Greenness Score (AMGS) calculator and the Analytical Greenness Metric (AGREE) software, showed that the proposed method has lower negative environment impact compared with the reference method. The method can be used in quality control of sorafenib film-coated tablets in the pharmaceutical industry, as well as in the authorized quality control laboratories, providing mitigation of the environmental pollution.

Key words: sorafenib, assay, ethanol, green method, HPLC, method validation

Introduction

The concept of "green analytical chemistry" (GAC) refers to the minimization and/or elimination of hazardous chemicals, reduction or replacement of toxic reagents, elimination or reduction of chemical waste, less energy consumption, and increased safety for the operator. GAC principles play a crucial role in the development strategy of modern pharmaceutical industry (Eldin et al., 2016). The reversed-phase high-performance liquid chromatography (RP-HPLC) is the predominant technique used in the quality control of medicines. However, this technique employs substantial amounts of organic solvents as eluents in the mobile phase. Acetonitrile, methanol, and tetrahydrofuran are among the most frequently used reagents in HPLC methods, although they are toxic to the environment (Nakov et al., 2023). Tyrosine kinase inhibitors (TKI) belong to a group of pharmacologic agents that use different inhibition modes of the protein kinases signal transduction pathways. The interest in protein kinase inhibitors began with the approval of the tyrosine kinase inhibitor (TKI) imatinib in United States of America 2001, by the Food and Drug Administration (FDA) (Thomson et al., 2023). Since then, based on their pharmacological inhibition more than thirty kinase inhibitors have been

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approved for clinical use in cancer therapy and other diseases.

Sorafenib (Fig. 1) is an active pharmaceutical ingredient (API), acting as a potent protein kinase inhibitor that targets proteins in cancer cells and stops the cancer cells from growing. It is used for the treatment of liver cancer, kidney cancer, and thyroid cancer. Sorafenib blocks the enzyme RAF kinase, a critical component of the RAF/MEK/ERK signaling pathway that controls cell division and proliferation; sorafenib inhibits the VEGFR-2/PDGFR-beta signaling cascade, thereby blocking tumor angiogenesis (Sankar et al., 2021).

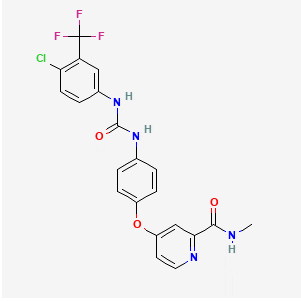

Fig. 1. Structure of sorafenib (NCBI, 2024).

There are several methods of analysis of sorafenib described in the literature based on UV/VIS spectrophotometry, vibrational spectroscopy, nuclear magnetic resonance spectrometry (^{1}H and ^{13}C NMR), and mass spectrometry, electrochemistry or chromatography (Abdelgalil et al., 2019). Considering that the chromatographic methods of analyses, including thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (European Pharmacopoeia, 2022; Sharma et al., 2021), are mostly used for pharmaceutical analysis of sorafenib, and that these methods use high amount of solvents that are environmental hazards, the aim of our study was to develop a green RP-HPLC method for determination of sorafenib in pharmaceutical formulations, using ethanol as an organic component of the mobile phase.

Material and methods

Chemicals and standards

Sorafenib tosilate (CRS Ph. Eur. Reference Standard, 99.7 %) was used as a reference substance. Sorafenib 200 mg film-coated tablets were used as a sample. Ethanol absolute and sodium dihydrogen phosphate anhydrous (99.99%) were purchased from Merck (Darmstadt, Germany). Water was purified by Direct-Q® 5 UV, Millipore® water purification system, obtained in-house at the Institute of Public Health - Skopje, Republic of North Macedonia. Nylon Membrane Filter (25 mm, 0.45 µm pore size) were purchased from Labfil (Zhejiang, China).

Instrumentation

HPLC analyses were performed on a Shimadzu Nexera-i LC-2040 SD Plus, integrated high-performance liquid chromatography system (Shimadzu Co.; Kyoto, Japan) with DAD detector. Instrument control, data collection and data processing were carried out using LabSolution software Version 5.99.

Chromatographic conditions

The separation was performed on a LichroCART® 60 RP-select B column (125 mm × 4 mm, 5 µm particle size, Merck KGaA, Darmstadt, Germany), using a mixture of 0.056 M sodium dihydrogen phosphate buffer pH 6.0 and ethanol in a ratio of 55:45 (% *V/V*) as a mobile phase, with isocratic elution and a flow rate of 1.0 mL/min within a run time of 6 minutes. The column temperature was maintained at 30ºC, and the auto-sampler was set to room temperature. The injection volume was 20.0 µL and the detection was set to 265 nm. A mixture of acetonitrile, water and ethanol in a ratio of 10:20:70 % *V/V/V* was used as a solvent for preparation of standard and sample solutions.

Standard solution

The standard solution was prepared by dissolving an amount (around 13.7 mg) of sorafenib tosilate CRS equivalent to 10 mg sorafenib, in a solvent, followed by treatment on an ultrasonic bath and dilution with the same solvent up to 100.0 mL (working concentration of sorafenib: 0.1 mg/mL).

Test solutions

Twenty randomly selected tablets were powdered in a dry and clean mortar. An amount of fine powder corresponding to a 10 mg sorafenib (about 16 mg tablet mass) was accurately weighed, transferred to a 100 mL volumetric flask, treated on an ultrasonic bath after adding approximately 70 mL solvent and diluted to the mark withthe same solvent. The standard and test solutions were filtered through 0.45 µm membrane filters (nylon filter) and vacuum degassed before use.

Method validation

The proposed method was validated according to the guideline for the validation of the analytical procedures established by the International Conference on Harmonization (ICH guideline Q2 (R2), 2022). The parameters used for the method validation analysis included system suitability, specificity, linearity, range, accuracy, and precision and robustness.

Reference method

The method for assay of sorafenib described in the monograph for Sorafenib tablets 01/2022:3022, published in the European Pharmacopoeia (Ph. Eur.) was used as a

reference method (European Pharmacopoeia, 2022). The chromatographic conditions of the Ph. Eur. method included use of an end-capped octadecylsilyl silica gel for chromatography R (100 mm × 4.6 mm, 3.5 µm) column as a stationary phase, with a gradient elution. The column used for separation was Symmetry C18 (100 mm × 4.6 mm, 3.5 µm particle size, Waters, USA). Mobile phase A consisted of anhydrous ethanol R, acetonitrile for chromatography and potassium dihydrogen phosphate buffer pH 2.4 (16:24:60 *V/V/V*), whereas mobile phase B consisted of anhydrous ethanol R and acetonitrile (40:60 *V/V/V*). The flow rate was 1.5 mL/min. The column temperature was maintained at 40 ºC. The injection volume was 10 µL and analyte peaks were monitored at a wavelength of 235 nm. The auto-sampler temperature was maintained at room temperature. Under these proposed conditions, the analysis time was 14 minutes.

Standard solution (Reference method)

The standard solution was prepared by dissolving 22 mg Sorafenib tosilate CRS in a solvent mixture of water R previously adjusted to pH 2.4 with phosphoric acid R and mobile phase B (25:75 *V/V*), followed by treatment on an ultrasonic bath and dilution with the same solvent up to 100.0 mL (working concentration: 0.16 mg/mL).

Results and discussion

Method development

A simple green method for the determination of sorafenib in film-coated tablets using the principles of GAC was developed, based on the modification of the reference method described in the Ph.Eur. monograph for sorafenib tablets. Three different reversed-phase columns were used for the method development (InterSustain C-8 150 mm x 3.9 mm, 5 µm, LiChrospher® 100 RP-8, 5 µm LiChroCART® 250 mm x 4 mm and LichroCART® 60 RP-select B column 125 mm × 4 mm, 5 µm). The main challenge was to remove the acetonitrile used in the mobile phase described in the reference method and replace it with ethanol. Ethanol has a low chemical reactivity, low vapor pressure, and higher elution power compared to acetonitrile and methanol. In addition, ethanol is less toxic, less flammable and with lower environmental impact (Nakov et al., 2023). Considering the estimated pKa values of sorafenib (strongest acidic pKa 11.55 and strongest basic pKa 3.03) (DrugBank, 2024), it was assumed that at any pH value above 5, sorafenib would exist in a non-ionized form. Therefore, the initial mobile phase used in method development composed of ethanol and water (60:40, *V/V*), but no satisfactory reproducibility of the sorafenib peak retention time was achieved. The water in the mobile phase was replaced with an acetate buffer pH 5.6, selected as an eco-friendly buffer, but the symmetry of the sorafenib peak was not satisfactory. The most satisfactory results for system suitability (Table 1) were obtained using the 60 RP-select B column and a mobile phase composed of ethanol and a phosphate buffer pH 6.0 (60:40, *V/V*). Concerning the temperature of the column, increasing the temperature from 25 to 30 °C, provided better peak shape and peak symmetry. The wavelength of 265 nm was chosen, based on the literature data for the wavelength of maximum absorption of sorafenib (265.5 nm) (Ravisankar et al., 2019) and experimental data (Fig. 2).

Table 1. Summary of the results for the system suitability parameters

Parameters	Proposed green method	Reference method
Retention time (min)	3.9 min	4 min
k’	3.6	6.0
Peak symmetry	1.05	1.24
Number of Theoretical plates	2007	9008
System precision, RSD (n=6)	0.59%	0.48%

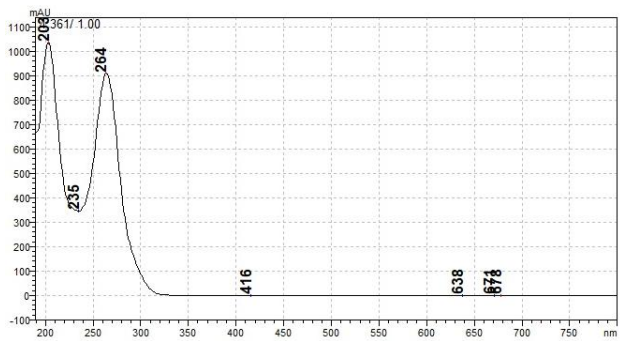

Fig. 2. UV spectrum of sorafenib obtained from the chromatogram of standard solution.

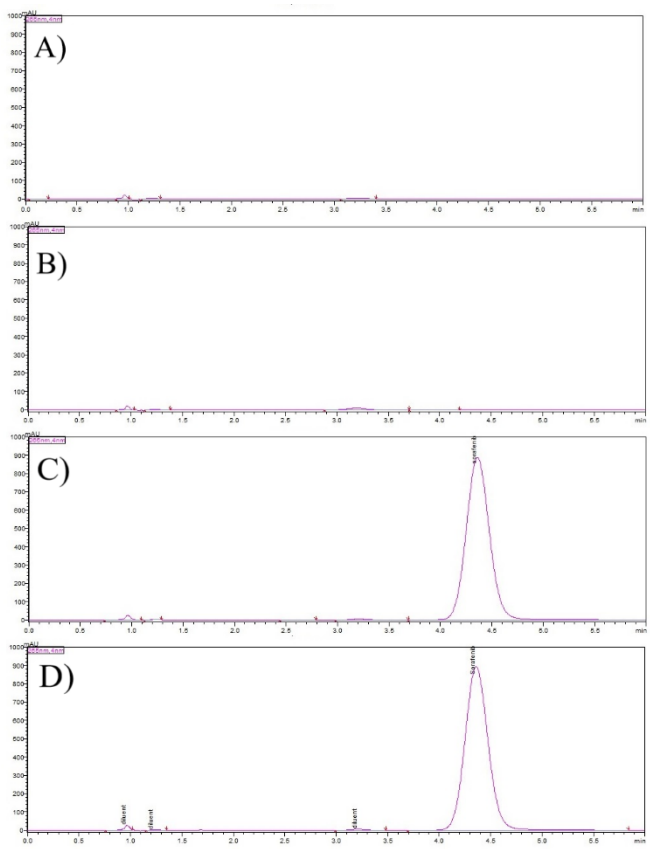

Fig. 3. Representative chromatograms of solvent (A), placebo (B), standard solution (C) and test solution for determination of assay of sorafenib (D).

The results from determination of the system suitability parameters obtained with the proposed green RP-HPLC method were compared with the results obtained using the method for assay of sorafenib described in the monograph Sorafenib tablets 01/2022:3022 published in the Ph.Eur as a reference method (European Pharmacopoeia, 2022). The results summarized in Table 1 show that the criteria for the system suitability of the method are fulfilled. The number of theoretical plates obtained from the proposed ethanol-based method are lower compared to the reference method, but the value is considered acceptable (N>2000).

Method validation

The proposed method was validated according to the guideline for the validation of the analytical procedures established by the International Conference on Harmonization (ICH Guideline Q2(R2), 2022). The parameters used for the method validation analysis included system suitability, specificity, linearity, range, accuracy, and precision and robustness.

Specificity of the method was confirmed from the chromatograms obtained after injections of the solvent, placebo standard and sample solution (Figure 3). From the chromatograms it is evident that there are no interfering peaks from the solvent or placebo with the peak of sorafenib. The peak from the tosylate is eluting at retention time around 1 minute, but due to the lack of absorption at 265 nm the peak is not detected in the chromatograms from standard and sample solutions.

The linearity of the method was evaluated in the range between 0.05 and 0.15 mg/mL for sorafenib (corresponding to the 50-150% of the test concentration of sorafenib). Results were evaluated by linear regression analysis using the least squares regression method. The value of the correlation coefficient 0.9999 ($y = 98528x + 34707$) confirms the linear relationship between the concentration of sorafenib and the corresponding peak area.

The method precision was estimated from the results obtained from the tests for the repeatability (system repeatability and method repeatability) and intermediate precision. The values for the RSD (n=6) for repeatability of the system (0.59%) and repeatability of the method (0.63%) were satisfactory as well as the values for intermediate precision (comparison of the results obtained in two days, two different HPLC system) with F value of 1.27 that was less than F critical value (5.05, calculated for 12 determinations). Overview of the results for the method precisions are presented in Table 2.

Table 2. Results for the method precision

Method repeatability	*RSD* = 0.63%*
Intermediate precision	
Analyst 1	RSD* = 0.63 %
Analyst 2	RSD* = 0.65 %
F - value	1.27
F critical (n = 12)	5.05

*$_{N=6}$

The accuracy of the proposed analytical method, was evaluated from recovery values of the determinations of sorafenib, using the standard additions method. The recovery values (Table 3) for sorafenib were between 99.74% and 99.97% indicating that the developed method is accurate and can be applied for the determination of sorafenib in pharmaceutical formulation. The results for accuracy are presented in Table 3.

The robustness of the method was confirmed from the results (Table 4) from system suitability parameters (peak symmetry, theoretical plates and retention time) obtained after varying the mobile phase flow rate (0.9-1.2 mL/min), column temperature (25-35 ºC), detection wavelength (263 - 267 nm) and the pH value of the buffer in the mobile phase (5.8-6.2), identified as potential factors that could affect the robustness of the method.

Assessment of the greenness features of the proposed method

The Analytical Method Greenness Score (AMGS) and the Analytical Greenness Metric (AGREE) approach were used to assess the greenness of the method. The greenness features of the proposed ethanol-based method were compared with the Ph. Eur method chosen as a reference method. The results obtained with the AMGS calculator (Hicks et al., 2019) are presented in Fig. 4. The calculated AMGS index for the Ph. Eur. reference method was 3283.66, while for the proposed ethanol-based method is 944.37. Lower values for the AMGS index indicate that the method has a better greenness score. The proposed green method has around 3.5 times better AMGS index in relation to the reference method. In addition, the evaluation showed that the proposed method offers a significant improvement in terms of “Instrument energy score”, “Solvent energy score” and “Solvent EHS score”, which is a result of the shorter run-time and exclusion of acetonitrile from the mobile phase.

The proposed and the reference methods are also compared using the AGREE tool (Pena-Pereira et al., 2020). The AGREE software is a tool for evaluating the environmental and occupational hazards associated with a particular analytical procedure based on the 12 principles of GAC. The results from the assessment were translated into a pictogram providing easy interpretation. The pictogram contains an overall score, as well as an indication to what degree the evaluated methods conform to each of the 12 GAC principles. These results can be used for comparison of two different methods, providing basis for selection of the method with the lowest environmental impact or to identify the most critical aspects related to greenness off the method during development of new analytical procedures. The results of the AGREE Calculator for both methods are shown in Figure 5. Considering that both methods are off-line methods, we obtained red color in both AGREE pictograms (non-compliance of the methods) for principle 3, as expected. Deviations from the GAC principle 7 and principle 11 (red color in both pictograms) were observed for both methods due to the use of large amount of waste generated by a single analyte determination and due to the use of the toxic reagent acetonitrile (the acetonitrile consumption in the reference method is larger than in the ethanol-based method, which uses only 10% acetonitrile in the mobile phase, the rest is completely replaced by ethanol). Deviation from the GAC principle 1 (expressed as an orange color in the pictograms of both methods) is due to the use of external sample pretreatment. For principle 5, the pictogram of the proposed method has a yellow color vs orange color in the pictogram of the reference method as the proposed method requires less reagents, solvents, and energy, although both methods are not fully automated. Orange color in the pictogram of the reference method for the principle 8 vs green color in the pictogram of the proposed method could be explained with a longer run time of the reference method (14 min vs 6 min); fewer runs can be analyzed during an hour as compared with the ethanol-based model. Both pictograms have a yellow color for principle 10 because both methods use one of the reagents that is derived from bio-based sources. The difference is that the reference method uses a much smaller amount of ethanol than the green method.

The results for the greenness assessment, obtained with the AGREE software, showed that the proposed method has higher ARGEE score (0.63) indicating better compliance with the GAC principles, compared with the reference method (ARGEE score of 0.46).

Table 3. Summary of the results from testing of the accuracy of the proposed method

Concentration level %	Recovery ± confidence interval (95% level of confidence)	RSD (n=3)
50	99.82±0.73%	0.29%
100	99.97±0.07%	0.03%
150	99.74±0.05%	0.02%

Table 4. Results from testing the robustness of the proposed method

Parameter		Retention time	Peak Symmetry	Theoretical plates
pH of the buffer	5.8	3.856	1.07	2003
	6.2	3.846	1.18	1989
Column temperature	25 ºC	4.094	1.09	1984
	35 ºC	3.583	1.10	2006
Wavelength	263 nm	4.141	1.09	2002
	267 nm	3.885	1.09	1985
Flow rate	0.9 mL/min	4.281	1.09	2007
	1.2 mL/min	3.589	1.09	2004

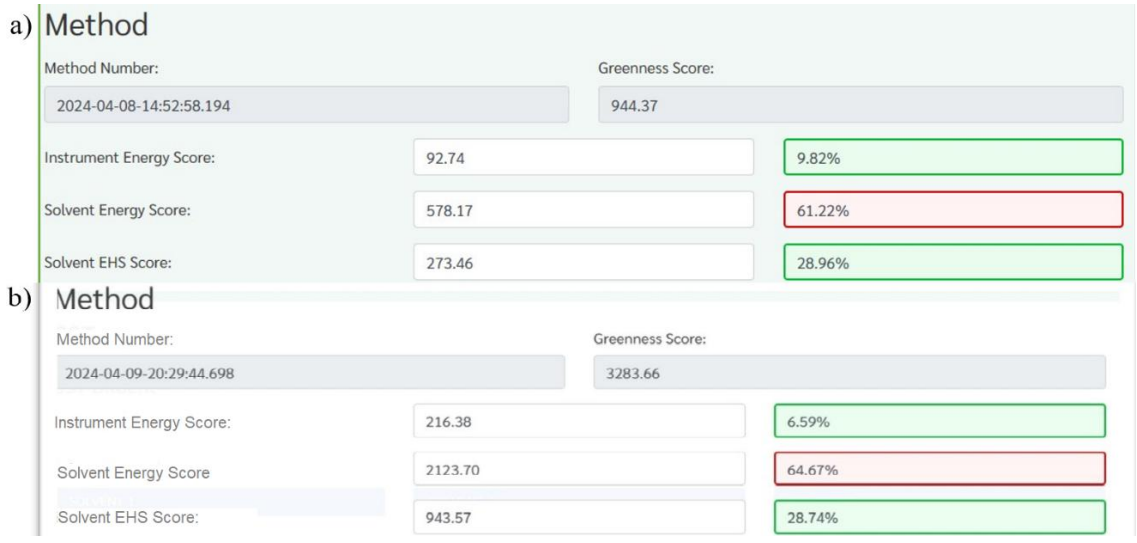

Fig. 4. Comparison of the developed method (a), reference Ph.Eur. Method (b) using AMGS tool.

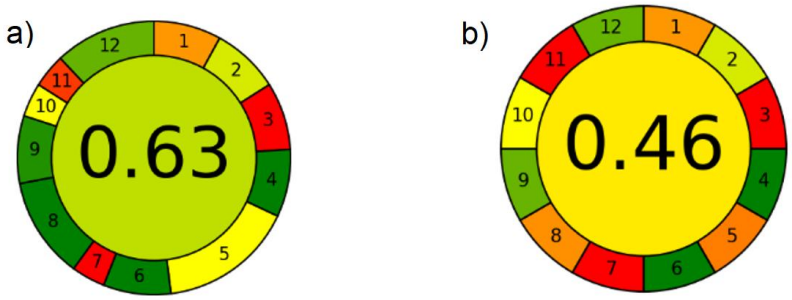

Fig. 5. Comparison of the developed method (a), reference Ph.Eur. Method (b) using AGREE tool.

Conclusion

A green, fast and simple RP-HPLC method for determination of sorafenib in film-coated tablets was developed and validated. The validation parameters confirmed that the method is specific, linear, precise, accurate and robust, and generates valid and reliable results, comparable to the reference method. The results for the greenness assessment, obtained with the AMGS calculator and the AGREE software, demonstrated that the method conforms to the GAC principles and has a lower negative environmental impact, compared with the reference method. Therefore, the method can be used in quality control of sorafenib film-coated tablets in the pharmaceutical industry, as well as in the authorized quality control laboratories, providing mitigation of the environmental pollution.

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Резиме

Развој на зелен RP-HPLC метод за определување на сорафениб во филм-обложени таблети

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Клучни зборови: сорафениб, определување на содржина, етанол, зелен метод, HPLC, валидација на метод

Развиен е и валидиран нов „зелен“ метод базиран на реверзно фазна хроматографија за определување на содржина на сорафениб во филм-обложени таблети. Хроматографското разделување е извршено на LichroCART® 60 RP-select B колона, со изократско елуирање, со примена на мобилна фаза составена од фосфатен пуфер со pH 6,0 и етанол во однос 45:55 (*V/V*), а температурата на колоната беше одржувана на 30 ºC. Брановата должина на детекција беше подесена на 265 nm. Методот е валидиран во согласност со ICH водичот, преку испитување на специфичноста, линеарноста, точноста, прецизноста и робусноста на методот. Резултатите добиени од проценката на ранг на еколошка подобност и зелен индекс на аналитички метод покажуваат дека методот е многу еколошки поприфатлив од референтниот метод. Предложениот метод обезбедува намалување на загадување на околината и може да се користи за контрола на квалитет на сорафениб филм-обложени таблети, како во фармацевтската индустрија така и во рутинската контрола во овластени лаборатории.