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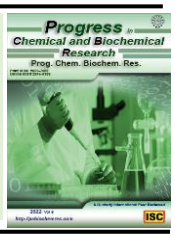

Original Research Article

Development and validation of RP-HPLC method for simultaneous quantification of the anticancer agents, nilotinib and sorafenib: Application in In-vitro analysis

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ABSTRACT

In this research, a reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of two tyrosine kinase inhibitors, nilotinib and sorafenib. Separation was performed on an Agilent C18 column (4.6×250 mm, 5µm) with mobile phase composition of potassium dihydrogen phosphate buffer (25 mM, pH 4.2) and acetonitrile (35:65 v/v) at 1.2 mL/min with UV detection at 265 nm. Specificity, linearity, precision, accuracy, and robustness of the proposed method were all assessed. Nilotinib and sorafenib had estimated retention times of 5.1 and 5.9 minutes, respectively. Linear concentration ranges for nilotinib and sorafenib, were determined as 0.05-1 µg/mL and 10-45 µg/mL with comparable coefficient correlations (0.999). For nilotinib and sorafenib, the limits of detection (LOD) were determined as 0.030 and 0.020 µg/mL, while the limits of quantification (LOQ) were 0.101 and 0.069 µg/mL respectively.

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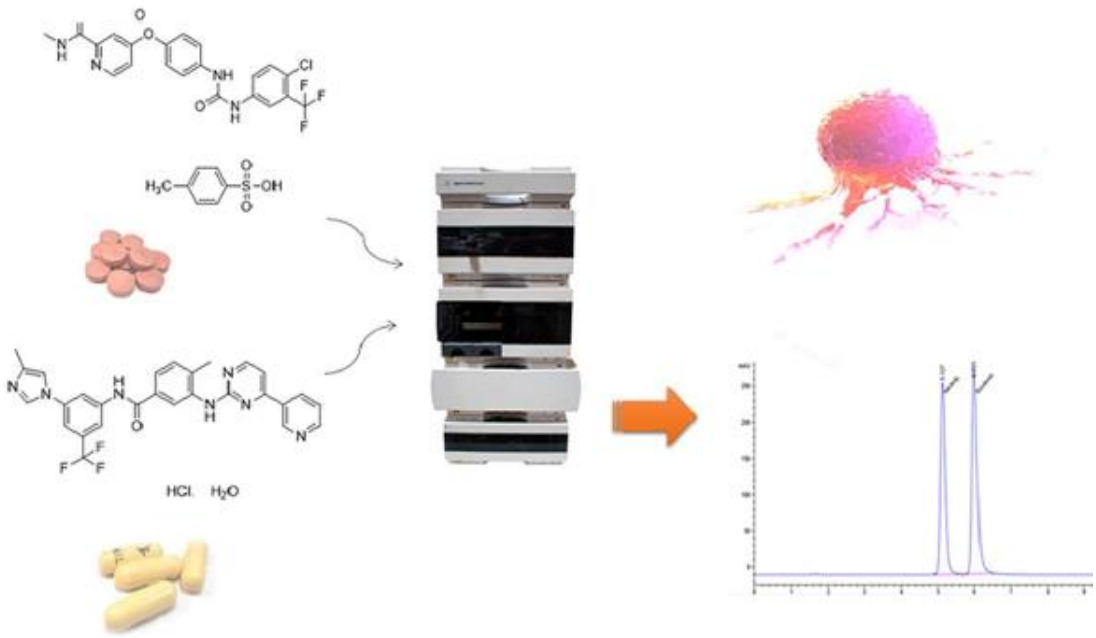
GRAPHICAL ABSTRACT



INTRODUCTION

Tyrosine kinase inhibitors (TKIs) are small-molecules which are often used to treat a variety of malignancies. According to earlier research, TKIs may enhance the long-term survival of patients in certain instances [1]. However, in some cases, such as chronic myeloid leukemia (CML) patients, response failure to such treatment are still seen [2]. Furthermore, like other anticancer drugs, the majority of patients suffer from severe side effects [3]. Nilotinib, 4-methyl-N-3-(3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl) phenyl)-3-(4-(pyridine-3-yl) pyramidin-2-ylamino) benzamide (Fig. 1), a second generation tyrosine kinase inhibitor is applied to treat the imatinib-resistant chronic myelogenous leukemia.

Nilotinib shows in vitro efficacy against several imatinib-resistant mutations and is selective for BCR-ABL, binding more than 20 times that of the affinity of imatinib [4-6]. Sorafenib tosylate, 4-[4-({[4-chloro-3- trifluoromethyl) phenyl] carbamoyl} amino) phenoxy] -N-methylpyridine-2-carboxamide (Fig. 2), is a multi-kinase inhibitor utilized in the colorectal cancer and hepatocellular carcinoma. As a monotherapy agent or in combination with other antitumor agents, sorafenib has indicated significant anticancer activity against different tumor types. In preclinical and clinical studies, it was proved that in addition to induction of apoptosis in tumor cells, sorafenib hinders cell proliferation as well as angiogenesis, [7-10].

Few analytical methods are available for the determination of nilotinib and sorafenib in bulk and pharmaceutical dosage forms [11-18]. Based on our investigation no analytical method has been reported for the simultaneous determination of nilotinib and sorafenib.

Pharmaceuticals, on the other hand, have recently been identified as a category of "emerging pollutants" in environmental contamination. Emissions from production, sewage leaks, direct dumping of unwanted medications, and human and animal excrement have all been linked to their presence in water samples [19]. As a result, determining the presence of drugs, particularly anti-cancer drugs, in the environment, such as water sources, could be beneficial. The current research was intended to create a simple, accurate, robust, and cost-effective HPLC method for simultaneous determination of

nilotinib and sorafenib in bulk, pharmaceutical dosage forms, and waste [20].

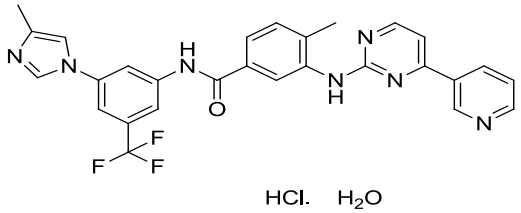


Fig. 1. Chemical structure of nilotinib hydrochloride monohydrate

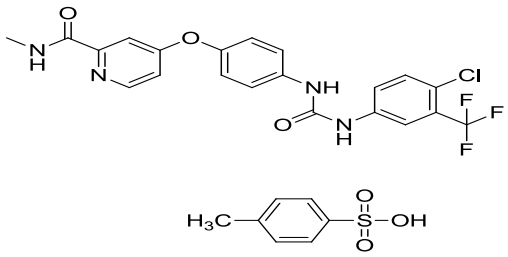


Fig. 2. Chemical structure of sorafenib tosylate

EXPERIMENTAL

Instrumentation

For the separation and determination of nilotinib and sorafenib, an HPLC system (Agilent Technologies, 1260 infinite quaternary LC VL) with a UV-visible detector was utilized. Agilent Chemstation software was used to collect chromatographic data.

Materials

Parsian Pharmaceutical Company, Tehran, Iran, generously supplied nilotinib hydrochloride monohydrate (Label claim 99.7% purity) and sorafenib tosylate (Label claim 99.1% purity). Noavaran Daroui Kimia Pharmaceutical Company, Tehran, Iran, donated the capsule dosage form of nilotinib (Kimonil 200 mg) and the tablet dosage form of sorafenib (Soranex 200 mg). All other chemicals and solvents were of analytical or HPLC grade including potassium dihydrogen phosphate, orthophosphoric acid and acetonitrile and purchased from Merck (Germany) and Duksan (Korea). Waste water sample was gathered from waste systems of two oncology pharmaceutical companies in Tehran.

Standard solutions

Nilotinib hydrochloride (500 µg/mL) and sorafenib tosylate (500 µg/mL) stock standard solutions were prepared in methanol. After dilution with the mobile phase, calibrating solutions in the range of 0.05 to 1 µg/mL and 10 to 45 µg/mL were prepared.

Chromatographic conditions

For chromatographic separation, an Agilent C18 column (Zorbax SB-C18 4.6×250 mm, 5µm) was utilized. The mobile phase was made up of a 35:65 v/v combination of potassium dihydrogen phosphate buffer (25 mM, pH=4.2) and acetonitrile. The mobile phase flow rate and UV detector set at 1.2 mL/min and 265 nm respectively. A 20 µL sample volume was injected and the separation took place at room temperature.

System Suitability

Six replicate injections of the standard solution (30 µg/mL) were used to test system suitability. Theoretical plate count and resolution were recorded as system suitability characteristics.

Accuracy and precision

Three separate series samples of nilotinib and sorafenib were produced within the calibration range (0.05 to 1 µg/mL and 10 to 45 µg/mL, respectively). The samples were analyzed by HPLC apparatus, and the concentrations of the samples were determined using the calibration curves. Within-day and between-day coefficient of variation (CV) and error were determined as indicators of method precision and accuracy.

Robustness

The method's robustness was tested by making modest adjustments to the elution flow rate, buffer pH, and organic phase composition of the mobile phase. In each experiment, one

parameter was modified based on the one factor at a time methodology.

Analysis of pharmaceutical dosage forms and waste water

Twenty sorafenib pills, each having 200 mg of active ingredient, were weighed and pulverized. A piece of the powder, approximately 25 mg of sorafenib, was carefully weighed and transferred to a 50 mL volumetric flask, to which 40 mL of methanol was added. The solution was sonicated for 3 minutes after producing the volume with the same diluent. Twenty nilotinib capsules (containing 200 mg active material) were precisely weighed, and a part of the powder, equal to approximately 25 mg of nilotinib, was precisely weighed and transferred to a 50 mL volumetric flask, to which 40 mL methanol was added. The solution was sonicated for 3 minutes after producing the volume with the same diluent. A 0.600 mL of each of the aforementioned solutions was transferred to a 10 mL volumetric flask and diluted to volume with diluent before being combined to achieve a final concentration of 30 µg/mL. Standard solutions of nilotinib and sorafenib were prepared at the same concentration level. Waste water samples were filtered with Whatman® filter paper 11µm pore size, then were injected to HPLC.

RESULTS

Chromatographic conditions

Various mobile phases with different organic phase and buffer compositions, as well as pH values, were used to find out the optimum condition and resolution for nilotinib and sorafenib. Sharp peaks of nilotinib and sorafenib with a resolution factor of approximately 3.3 were obtained using a 35:65 mixture of potassium dihydrogen phosphate buffer (25 mM, pH=4.2) and acetonitrile at a flow rate of 1.2 mL/min. (Fig. 3).

System Suitability

System suitability parameters were computed after six replicate injections and are mentioned in Table 1.

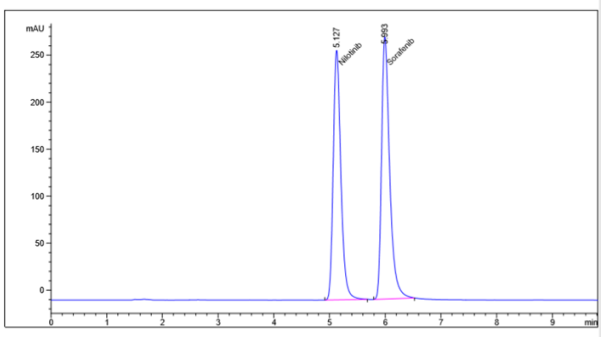


Fig. 3. Representative chromatogram of working solutions of nilotinib and sorafenib (30 µg/mL)

Linearity

Calibration graphs for sorafenib and nilotinib were produced in the ranges of 0.05-1 and 10-45 µg/mL. For both nilotinib and sorafenib, good linearity was found in terms of correlation coefficients ≥ 0.99. The values for limit of detection and limit of quantification were determined and are presented in Table 2.

Accuracy and precision

Tables 3 and 4 demonstrate the results of the examination of three separate standard solutions of sorafenib and nilotinib within a day and between three different days, demonstrating adequate accuracy and repeatability.

Robustness

The proposed method's robustness was tested under the various chromatographic settings described in the experimental section. No significant changes in peak area, shapes or resolution of nilotinib and sorafenib peaks were detected with modest adjustments in flow rate (1.2±0.1 mL/min) and pH of mobile phase (4.2±0.2). The peak area did not vary much when the organic solvent proportion in the mobile phase (35±2 %) was changed. Some minor changes were observed in resolution,

although they were still within acceptable limits. The results of this test indicate the robustness of method.

Table 1. System suitability parameters

Parameters	Nilotinib	Sorafenib	Acceptable limits
USP theoretical plates (n=6)	7800	8100	N>1500
USP tailing factor (n=6)	0.9	0.8	T<1.5
Repeatability (t_R) (n=6)	0.147	0.161	RSD<1%
Repeatability (peak area) (n=6)	0.140	0.344	RSD<1%

tR: Retention time (min); N: Theoretical plate; T: Tailing factor; RSD: Relative Standard Deviation

Table 2. Statistical data of calibration curves of nilotinib and sorafenib (n=6)

Parameters	Nilotinib	Sorafenib
Linearity range (Low)	0.05-1 (µg/mL)	0.05-1 (µg/mL)
Linearity range (High)	10-45 (µg/mL)	10-45 (µg/mL)
Regression equation (Low)	y=82.91x-0.09	y=97.61x-0.31
Regression equation (High)	y=83.81x-10.41	y=96.00x+5.54
Standard deviation of slope (Low)	0.625	0.506
Standard deviation of slope (High)	0.412	0.564
Standard deviation of intercept (Low)	0.321	0.260
Standard deviation of intercept (High)	12.20	16.71
Correlation coefficient (r^2)	0.999	0.999
LOQ	0.101 (µg/mL)	0.069 (µg/mL)
LOD	0.030 (µg/mL)	0.020 (µg/mL)

Table 3. Accuracy and precision analysis data of the method for the determination of nilotinib and sorafenib (0.05-1 µg/mL)

Added (µg/mL)	Within-day (n = 3)			Between-day (n = 9)		
	determined (µg/mL)	CV (%)	Error (%)	determined (µg/mL)	CV (%)	Error (%)
Nilotinib						
0.250	0.251±0.001	0.44	0.40	0.252±0.002	0.74	0.80
0.500	0.504±0.003	0.59	0.80	0.504±0.003	0.59	0.80
0.750	0.751±0.001	0.20	0.13	0.751±0.001	0.18	0.13
Sorafenib						
0.250	0.249±0.002	0.09	0.40	0.252±0.002	0.89	0.80
0.500	0.501±0.002	0.43	0.20	0.501±0.002	0.46	0.20
0.750	0.752±0.001	0.12	0.27	0.752±0.002	0.31	0.27

Table 4. Accuracy and precision data of the determination of nilotinib and sorafenib (10-45 µg/mL)

Added (µg/mL)	Within-day (n = 3)			Between-day (n = 9)		
	determined (µg/mL)	CV (%)	Error (%)	determined (µg/mL)	CV (%)	Error (%)
Nilotinib						
15.00	14.99±0.04	0.29	0.07	15.01±0.08	0.56	0.07
30.00	30.22±0.03	0.08	0.73	30.09±0.15	0.51	0.30
45.00	45.01±0.12	0.27	0.02	44.90±0.19	0.43	0.22
Sorafenib						
15.00	15.12±0.04	0.26	0.80	15.05±0.13	0.85	0.33
30.00	30.02±0.06	0.18	0.07	29.82±0.22	0.75	0.60
45.00	45.09±0.24	0.53	0.20	44.92±0.31	0.70	0.18

Table 5. Comparison of some HPLC methods for the analysis of nilotinib or sorafenib

Ref.	**Analyte**	**Column**	**Mobile phase and mode**	**Detector**	**tR (min)**	**LOQ (µg/mL)**	**Linearity range (µg/mL)**
11	Nilotinib	C18 (150 × 4.6 mm, 3.5 µm)	Ammonium formate (pH 3.5)-acetonitrile, gradient	PDA	4.37	--------	0.04-3.0
13	Sorafenib	C18 (250 × 4.6 mm, 5 µm)	Methanol-water (80:20, v/v), isocratic	UV	4.52	0.45	20-100
14	Nilotinib	C18 (150 x 4.6 mm, 5µm)	Acetonitrile-phosphate buffer (pH 5) (60:40, v/v), isocratic	PDA	5.40	0.03	2-10
15	Nilotinib	C18 (250 × 4.6 mm, 5 µm)	Methanol-acetonitrile-water (30:20:50, v/v), isocratic	UV	4.63	0.22	2-10
16	Sorafenib	C 18 (150 X 4.6 mm, 5 µm)	Acetonitrile-phthalate Buffer-methanol (75: 24.5: 0.5, v/v) (pH 4), isocratic	UV	6.30	0.017	0.05 – 2.0
17	Imatinib Sorafenib	C18 (250 x 4.6 mm, 2 µm)	Ammonium acetate buffer (pH 8.5)-methanol (35:65, v/v), isocratic	UV	1.2 3.8	0.63 0.63	1 – 16 1-16
18	Sorafenib Paclitaxel	C18 (250 × 4.6 mm, 5 µm)	Acetonitrile-TFA (0.025%) (65:35, v/v), isocratic	UV	7.41 5.55	0.010 0.015	0.01-5 0.015-20
**	Nilotinib Sorafenib	C18 (250 × 4.6 mm, 5 µm)	Potassium dihydrogen phosphate buffer (pH 4.2)-acetonitrile (35:65, v/v), isocratic	UV	5.10 5.90	0.101 0.069	0.05-1.00 and 10-45 for both

**Present study

Analysis of pharmaceutical dosage forms and waste water

Nilotinib and sorafenib in their pharmaceutical dose forms were effectively determined using the suggested validated technique. The findings for nilotinib and sorafenib were 100.73% and 100.06% similar to the indicated levels, respectively. Because of the appropriate limit of detection (0.030 and 0.020 µg/mL) and limit of quantification (0.101 and 0.069 µg/mL) for nilotinib and sorafenib, respectively, this validated technique was used to detect these anticancer medicines in waste water.

DISCUSSION

The chromatographic parameters were adjusted to create a RP-HPLC technique for the simultaneous determination of nilotinib and sorafenib in pharmaceutical dosage forms and wastewater samples with a fast analysis time (<7 min) and adequate resolution (Rs> 3). Various mobile phase compositions were investigated. The resolution by using phosphate buffer (25 mM, pH=4.2) and acetonitrile (35: 65 v/v) as the mobile phase was excellent. Both substances showed linearity in the concentration ranges of 0.05-1 µg/mL and 10-45 µg/mL, with a correlation coefficient of 0.999. Limits of detection (LOD) for nilotinib and sorafenib were 0.030 and 0.020 µg/mL, respectively, while limits of quantification (LOQ) were 0.101 and 0.069 µg/mL based on calibration curves analysis. The technique was determined to be accurate and precise since the recovery values were within acceptable limits and the CVs were less than 2.0 percent. There are few reported HPLC methods for the determination of sorafenib or nilotinib alone or in the presence of other compounds. The comparison of these methods with our method is presented in Table 5. In our study simultaneous determination of sorafenib and nilotinib is described for the first time. The limit of quantification of our method is better or comparable with the previously reported methods. The main advantage of our method is simultaneous determination of two drugs in a total run time of 7 min. Also two validated calibration ranges could be used for the analysis of sorafenib and nilotinib in a wide range of concentration.

CONCLUSION

For the simultaneous measurement of nilotinib and sorafenib in bulk, pharmaceutical dosage forms, and waste water samples, a validated RP-HPLC technique was devised. Using these analytical settings, good resolution was achieved in a short time. The suggested technique may be utilized for the simultaneous analysis of nilotinib and sorafenib in pharmaceutical dosage forms in labs and for quality control reasons since it is simple, fast, accurate, precise, and specific. Another benefit of this technique is its sensitivity, as shown by the low LOD and LOQ values obtained for nilotinib and sorafenib. As a result, the technique may be useful in applications such as environmental and bioanalysis.

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REFERENCES

[1] M. A. Ali, Chronic myeloid leukemia in the era of tyrosine kinase inhibitors: an evolving paradigm of molecularly targeted therapy. Molecular Diagnosis & Therapy, 20 (2016) 315–333.

[2] J. M. Diamond, J.V. Melo, Mechanisms of resistance to BCR-ABL kinase inhibitors. Leukemia & Lymphoma, 52 (2011) 12–22.

[3] T. Lahaye, B. Riehm, U. Berger, P. Paschka, M.C. Müller, S. Kreil, K. Merx, U. Schwindel, C. Schoch, R. Hehlmann, A. Hochhaus, Response and resistance in 300 patients with BCR-ABL-

positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. Cancer, 103 (2005) 1659–1669.

[4] P. W. Manley, P. Drueckes, G. Fendrich, P. Furet, J. Liebetanz, G. Martiny-Baron, Extended kinase profile and properties of the protein kinase inhibitor nilotinib. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1804 (2010) 445-453.

[5] E. Weisberg, P. Manley, J. Mestan, S. Cowan-Jacob, A. Ray, J. D. Griffin. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. British Journal of Cancer, 94 (2006) 1765-1769.

[6] J. Y. Blay, M. von Mehren, Nilotinib: A novel, selective tyrosine kinase inhibitor. Seminars in Oncology, 38 (2011) S3-S9.

[7] A. R. He, A. S. Goldenberg, Treating hepatocellular carcinoma progression following first-line sorafenib: therapeutic options and clinical observations. Therapeutic Advances in Gastroenterology, 6 (2013) 447-458.

[8] C. H. Takimoto, A. Awada, Safety and anti-tumor activity of sorafenib (Nexavar®) in combination with other anti-cancer agents: a review of clinical trials. Cancer Chemotherapy and Pharmacology, 61 (2008) 535-548.

[9] S. M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, C. Chen, S. Zhang, P. Vincent, M. McHugh, BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Research, 64 (2004) e70997109.

[10] J. W. Panka, D. J. Wang, W. Atkins, M. B. Mier, The Raf inhibitor BAY 43-9006 (Sorafenib) induces caspase independent apoptosis in melanoma cells. Cancer Research, 66 (2006) 1611–1619.

[11] R. Ivaturi, T. M. Sastry, S. Satyaveni, Development and validation of a stability indicating HPLC method for the determination of nilotinib hydrochloride in bulk and pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 8 (2016) 41-48.

[12] A. Barla, K. Buralla, Development and validation of method for the determination of nilotinib by RP-HPLC in bulk and pharmaceutical dosage forms. International Journal of Pharmaceutical Investigation, 3 (2013) 364-367.

[13] Q. Z. Rafeeque, P. S. Dabhade, Development and method validation of RP-HPLC method for simultaneous estimation and forced degradation of sorafenib tosylate in bulk and pharmaceutical dosage form. Innovational Journal of Chemistry, 1 (2016) 63-71.

[14] A. Barla, K. K. Buralla, Development and validation of RP-HPLC method for the estimation of nilotinib in bulk and pharmaceutical dosage form, International Journal of Pharmaceutical Investigation, 10 (2020) 364-367.

[15] A. Rade, K. Patil, T. Thorat, P. Patil and D. Shinde, RP-HPLC method development and validation for the estimation of nilotinib in bulk and tablet dosage form, World Journal of Pharmaceutical Research, 8 (2019) 1286-1294.

[16] W. Talaat, M. M.Y. Kaddah, Validated analytical method for the determination of sorafenib in dosage form and human plasma in presence of it degradation products, International Journal of PharmTech Research, 12 (2019) 08-21.

[17] H. A. Alhazmi, D. Allah Moraya, E. Alahdal, M. Kariri, M. A. Bratty, Z. Rehman, S. A. Javed, Ultrafast monolithic HPLC method for simultaneous quantification of the anticancer agents, imatinib and sorafenib: Application to tablet dosage forms, Tropical Journal of Pharmaceutical Research, 17 (2018) 1127-1134.

[18] I. Khan, Z. Iqbal, A. Khan, M. Hassan, F. Nasir, A. Raza, L. Ahmad, A. Khan, M. A. Mughal, A simple, rapid and sensitive RP-HPLC-UV

method for the simultaneous determination of sorafenib and paclitaxel in plasma and pharmaceutical dosage forms: Application to pharmacokinetic study, Journal of Chromatography B, 1033-1034 (2016) 261-270.

[19] I. Baranowska, B. Kowalski, Using HPLC method with DAD detection for the simultaneous determination of 15 drugs in surface water and wastewater. Polish Journal of Environmental Studies, 20 (2011) 21-28.

[20] ICH Guidelines Q2 (R1), Validation of analytical procedures: Text and methodology, Geneva, 2005.

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