

Development and validation of a HPTLC method for analysis of Sunitinib malate

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A simple high performance thin layer chromatography (HPTLC) has been developed and validated for determination of sunitinib malate and possible impurities. The samples were applied in forms of bands on an aluminum TLC plate pre-coated with silica gel and were separated using dichloromethane:methanol:toluene: ammonia solution as the mobile phase. Sunitinib malate was thoroughly separated from impurities including E-isomer, sunitinib *N*-oxide and impurity B with a retention factor (RF) of 0.35 ± 0.02 . Quantitative analysis of sunitinib was carried out using a mobile phase consisting of dichloromethane:methanol:ammonia solution, RF value was 0.53 ± 0.02 for Z isomer. Detection was performed densitometrically in absorbance mode at 430 nm. This method was found to produce sharp, symmetrical, and well resolved peaks. Linear relationship with the coefficients of determination > 0.99 was achieved over the concentration range of 27.34 to 437.5 ng/spot. This method provides robust, replicable and accurate results with acceptable sensitivity.

Uniterms: Sunitinib malate/quantitative analysis. High Performance Thin Layer Chromatography/method/validation. Sunitinib malate/E-Z isomer.

INTRODUCTION

Sunitinib malate, *N*-(2-diethylaminoethyl)-5-[(*Z*)-(5-fluoro-2-oxo-1*H*-indol-3-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide, is an oral anticancer drug, which is marketed under the trade name Sutent® by Pfizer, Inc., New York. The chemical structure of sunitinib malate is shown in Figure 1. This drug is a novel multitargeted tyrosine kinase inhibitor with antitumor and antiangiogenic activities for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST) (Kessler *et al.*, 2007).

A very important stage in the process of production and development of pharmaceuticals is the analysis of

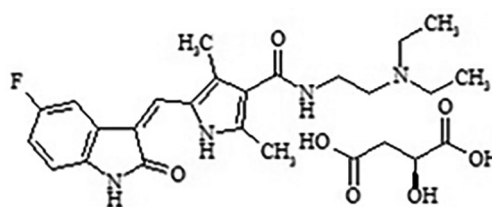


FIGURE 1 - Chemical structure of sunitinib malate.

components present in bulk drugs and their formulations in order to ensure that pharmaceutical products are of the quality required for their intended use. Accordingly so far, a few analytical methods have been established for the determination and quantitative analysis of sunitinib malate, which are mainly based on High Performance Liquid Chromatography (HPLC) method (Rizwana, Prakash, Mohan, 2014; Sandhya *et al.*, 2011). Furthermore, many methods mostly based on LC/MS have been presented in order to monitor drugs in biological fluids such as plasma (Oberoi, Mittapalli, Fisher, 2013;

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Etienne Grimaldi *et al.*, 2009; Minkin *et al.*, 2008; De Bruijn *et al.*, 2010). However, to the best of our knowledge, there is no published analytical method for detection and quantification of sunitinib malate based on thin layer chromatography (TLC/HPTLC).

Thin layer chromatography is a chromatography technique used to separate and analyze mixtures using a thin stationary phase supported by an inert backing. From the mid-1970s, with the introduction of modern methodological and instrumental advances made in TLC (called high performance TLC), the application of this method in qualitative and quantitative analysis of pharmaceutical, environmental, toxicological, food, and agricultural samples has increasingly grown (Sherma, 2010; Sherma, 2000, 2009; Le Roux *et al.*, 1992; Rakesh *et al.*, 2013). This trend continued so that nowadays in some quality control laboratories quantitative HPTLC is routinely used (Ferenczi-Fodor, Renger, Veigh, 2010).

The main advantages of HPTLC are as follows: simplicity of procedure, minimal pretreatment, efficiency with small amounts of sample, parallel analysis of samples (a 20×10 cm plate can contain up to 72 samples, which can be analyzed simultaneously and under identical conditions), multiple nondestructive methods for detection (for example visualization and scan in visible or UV light at different wavelengths), numerous options for developing solvents, low consumption of solvents, time and cost effectiveness (In fact, the expenses associated with providing solvents and maintenance are much lower in comparison with HPLC.) These advantages along with reliability, sensitivity, and reproducibility enable this method to be an important alternative to other chromatographic techniques such as HPLC. As an instance, the results of a study indicated that while the precision and accuracy of HPLC and HPTLC methods of phospholipids can be comparable, HPTLC was more cost-effective than HPLC (Renger, 1999).

In recent years, numerous publications have been devoted to the application of HPTLC methods in pharmaceutical analysis (Sherma, 2010; Askal *et al.*, 2008; Kadam, Bari, 2007; Mhaske, Dhaneshwar, 2007). For example, the following cases can be mentioned: determination of amantadine, an antiviral drug, in pharmaceutical formulations (Askal *et al.*, 2008), simultaneous analysis of valsartan and hydrochlorothiazide, an antihypertensive drug, in tablet formulation (Kadam, Bari, 2007), determination of omeprazole, a proton pump inhibitor, in capsule form (Jha *et al.*, 2010), simultaneous quantitation of paracetamol, diclofenac potassium, and famotidine in tablet formulation (Khatal *et al.*, 2010), determination of imatinib (Vadera, Subramanian, Musmade, 2007) and dasatinib (Mhaske, Dhaneshwar,

2007) as anticancer medicines in pharmaceutical form.

The present study describes a simple and viable analytical method for identification and quantitative analysis of sunitinib malate in bulk drug employing HPTLC-densitometric technique. The proposed method was developed and validated based on ICH guidelines.

MATERIAL AND METHODS

Material

Sunitinib malate salt was obtained from chemistry department at TODA Research & Engineering Company, Iran and was used without any purification. Standard of E and Z isomer mixture, sunitinib malate *N*-oxide and impurity B was purchased from TLC PharmaChem Inc., Canada. HPLC-grade methanol, dichloromethane, acetic acid, toluene, and ammonium hydroxide (25%) were purchased from Merck, Germany. All dilute solutions of ammonium hydroxide were prepared from demineralized water with a specific conductance equal to $1.2 \pm 0.1 \mu\text{Scm}^{-1}$.

Instrumentation

An automatic sample applicator (Camag Linomat IV, Switzerland) equipped with a Hamilton 100- μL syringe was employed for samples application on the HPTLC plate. Chromatographic separations were performed on 20×20 cm aluminum backed plates pre-coated with 0.2 mm layers of silica gel and fluorescent indicator with a 254 nm excitation wavelength (Kieselguhr 60F-254, E. Merck, Germany). A Camag twin-trough chamber for 20×20 cm plates, with a stainless steel lid, was used for ascending development of the plates. Densitometric scanning was performed on Camag TLC scanner II, operated with CATS3 software while the source of radiation was a tungsten lamp.

HPTLC analysis

Pre-conditioning: After selection of the chromatographic layer, plates were prewashed with methanol, and were then activated at 70 °C for 60 min.

Sample application: By means of an automatic applicator the samples were spotted with a constant application rate of 5 μL in the form of bands of 6 mm in width. The space between bands was 4 mm. The distance from the left edge and the bottom of the plate was kept at 30 and 20 mm respectively.

Selection of suitable mobile phase: The following solvent mixtures were selected as candidates for the method development: dichloromethane: methanol,

dichloromethane: methanol: acetic acid, dichloromethane: methanol: ammonia 25%-5%), dichloromethane: methanol: triethylamine, and dichloromethane: methanol: toluene: ammonia. Different compositions of above mentioned solvents were tested taking into account their polarities in order to select the optimized mobile phase for separation of sunitinib malate.

Chromatographic development: The tank was saturated for 15 min before insertion of the spotted plate. Plates were developed with 20 mL of mobile phase in strict light-protected conditions. The development distance was approximately 100 mm. The chromatography was run at 25 °C ± 3 and at relative humidity of 33% ± 3.

Detection and scanning: After development, the plate was dried in open air for 5 min. Densitometric scanning was then performed in the absorbance mode and at the speed of 4 mm/s, using the tungsten light source at 430 nm (λ_{max} for the compounds). Monochromator bandwidth was kept at 30 nm and the dimension of slit was set at 5 mm in length and 2 mm in width.

Preparation of stock and working solutions

A stock solution was prepared (500 µg/mL) by dissolving and diluting 5 mg of sunitinib malate (MM=532.561 g/mol) to 10 mL with methanol in light-protected conditions. The solution was sonicated for 15 minutes. Working solutions of sunitinib malate were prepared in amber colored glass vials by further dilution of the stock with methanol in a concentration range of 3.91 to 250 µg/mL.

Calibration curve

The amount of 7 µl of each working solutions (3.91 to 250 µg/mL), equivalent to 27.3, 54.6, 109.3, 218.7, 291.6, 437.5, 875.0 and 1750.0 ng per spot of sunitinib malate, were applied in three replicates on the TLC plate. The spotted plates were developed and scanned as described above. Calibration curve was constructed by plotting average peak areas versus the corresponding amounts and regression equation was calculated for sunitinib malate.

RESULTS AND DISCUSSION

Method Development and Optimization

Various chromatographic conditions were investigated to attain satisfactory results of sunitinib malate qualitative and quantitative analysis. Developing the mobile phase individually on glass and aluminum TLC plate and comparing results indicated that using aluminum backing plates produces well defined spots with better resolution. Hence, in this work, aluminum sheet plates precoated with silica gel were selected as stationary phase.

In order to achieve the optimal mobile phase which enables the separation of sunitinib malate and any possible impurities, different compositions and ratios of solvents with various polarities were tested. For instance, a number of solvent systems applied in this study are listed in Table I.

The most important impurities in this study were E isomer of sunitinib, sunitinib N-oxide (C₂₂H₂₇N₄O₃),

TABLE I - Different kinds of solvent mixtures which are tested in this study along with R_F values

Mobile phase	Volume ratio (v:v)	R _F values			
		Z-isomer	E-isomer	N-oxide	Impurity B
ethyl acetate: toluene: acetic acid	5:3:2	0	0	0	0
dichloromethane: methanol	9:1	0.6	0.56	0.6	1
	5:1	0.43	0.40	0.5	1
	3:1	0.32	0.22	0.30	1
	1:1	0.29	0.29	0.29	1
dichloromethane: methanol: acetic acid	3:1:0.05	0.41	0.33	0.51	1
dichloromethane: methanol: NH ₃ (25%)	3:1:0.1	0.37	0.37	0.39	1
dichloromethane: methanol: NH ₃ (8%)	3:1:0.05	0.40	0.31	0.41	1
dichloromethane: methanol: NH ₃ (5%)	3:1:0.1	0.53	0.42	0.64	1
dichloromethane: methanol: toluene: NH ₃ (5%)	3:1.5:0.5:0.05	0.35	0.25	0.47	0.94
dichloromethane: methanol: toluene: NH ₃ (5%)	3:1:1:0.05	0.43	0.36	0.46	0.86

and impurity B ($C_{20}H_{20}FN_3O_2$). Due to the presence of a double bond between 2-oxindol and the pyrrole ring, sunitinib has two stereoisomeric forms: the E and Z isomers (Honeywell *et al.*, 2010). Although Z isomer is the thermodynamically stable form, it can convert to E isomer when it is introduced into the solution exposed to light. Also, some studies have demonstrated that E isomer can convert to Z isomer when sunitinib malate was placed in the dark. Only Z isomer of sunitinib is a drug substance while E isomer is inactive and unstable. Hence, all experiments were done in light-protected conditions. However the Z-to-E isomerism of sunitinib could not be completely avoided. The results of trials showed that among the solvents which were tested a combination of dichloromethane and methanol with volume ratio of 3:1 perfectly separated the two stereoisomeric forms of sunitinib malate, so that E isomer appears (as a small peak) before Z isomer (desired drug substance) and produces sharp and symmetrical peaks without tailing. However, using this solvent system led to an overlap between the peak of sunitinib *N*-oxide and Z isomer. Hence, in another trial, ammonium hydroxide solution with different volume ratios (25% to 5%) was added to the above system. It was found that addition of 0.1 ml diluted ammonium hydroxide to 5% can completely separate main drug substance from E isomer and sunitinib *N*-oxide impurity. Under established conditions, quantitative analysis of sunitinib malate was carried out and the retention factor (R_f) value for Z-isomer of sunitinib was obtained, 0.53 ± 0.02 (Figure 2). Nevertheless, as the solvent system mentioned above develops, impurity B appears on the solvent front and hence could not be properly detected. After several trials, it was found that decreasing polarities of the mentioned solvent system by adding 0.5 mL of toluene leads to the separation of all considered components with satisfactory R_f values (Figure 3). Furthermore, the results showed that the saturation of chamber with mobile phase vapors for 15 min at room temperature before the development of the plate improves the spot characteristic.

Also, the optimum wavelength was determined to be 430 nm after scanning the developed plate from 400 nm to 700 nm using the tungsten lamp.

METHOD VALIDATION

The developed method was validated by linearity, accuracy, precision, robustness, limit of detection (LOD) and limit of quantitation (LOQ) according to the ICH guidelines. These will be discussed separately in the following sections.

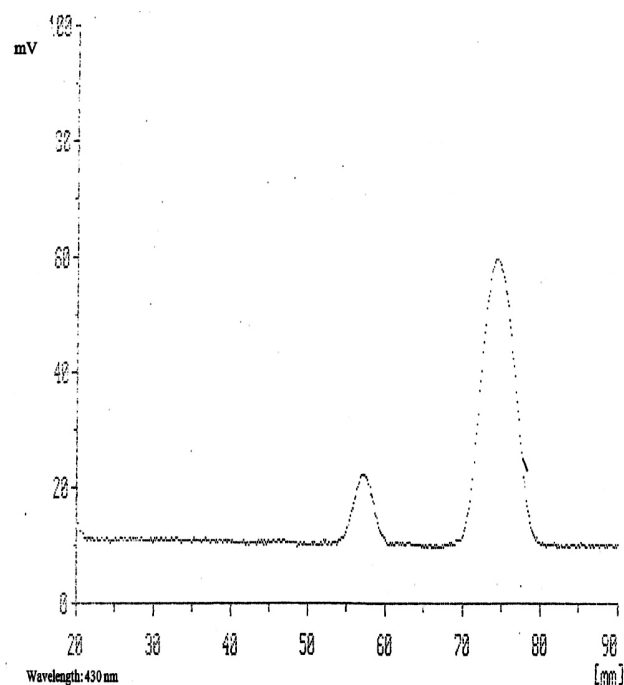


FIGURE 2 - A typical HPTLC chromatogram of sunitinib malate.

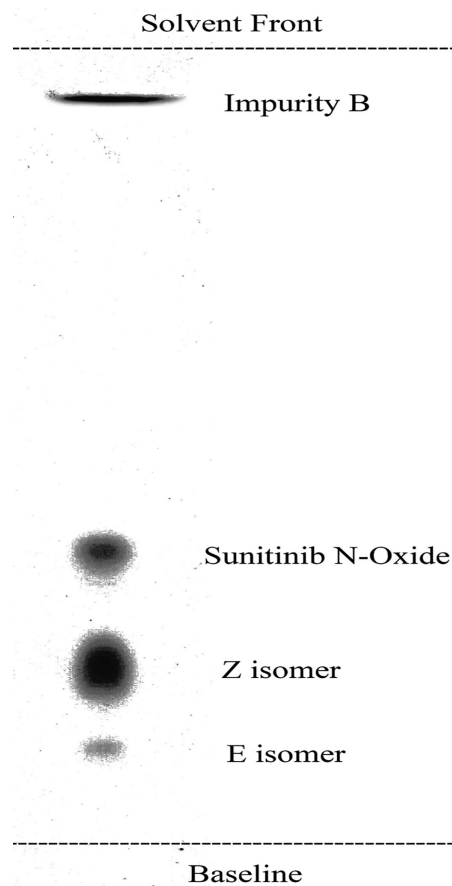


FIGURE 3 - Separation of Z isomer of sunitinib from related impurities using dichloromethane: methanol: toluene:NH₃(5%) with volume ratio of 3:1.5:0.5:0.05.

LINEARITY

To evaluate linearity range, the calibration curve ($n=3$) was plotted based on the peak area response versus sunitinib malate amount and was treated by linear least square regression (Figure 4). The results as presented in Table 2 showed a good linear relationship ($R^2=0.997$) over the concentration range 27.34- 437.5 ng per spot with respect to peak area.

TABLE II - Linear regression data for the calibration curve^a

Parameters	Amounts
Linearity range	27.34 - 437.50 ng per spot
R^2	0.997
slope	4.0191
Intercept	78.5292

^a $n=3$

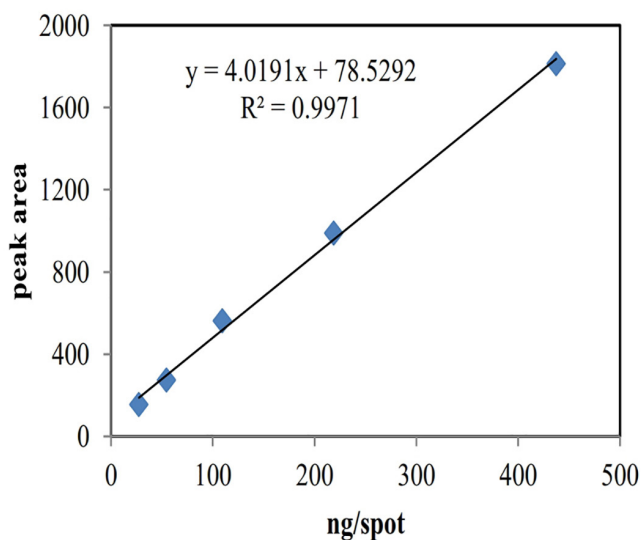


FIGURE 4 - Calibration curve for sunitinib malate.

Precision

The precision was estimated at three different concentration levels of 54.69, 218.75, and 291.67 ng per spot to evaluate three sources of variation. At first, precision was assessed when the same spot is measured three times by densitometer. Secondly, the same solution is spotted three times, and finally overall repeatability was evaluated when the analysis was carried out in the same conditions by the same analyst during two different days. The results presented as relative standard deviation percentage (%RSD) depicted in Table III. The obtained

RSD values were less than 3%, which confirms the good repeatability of sample application and measurement of peak area as well as acceptable intermediate precision. In TLC densitometry, RSD value in range of 1%–5% is acceptable (Komsta *et al.*, 2013).

TABLE III - Precision data for sunitinib malate^a

Parameters	S.D.	%RSD
measurement precision	7.88	0.75
spotting precision	13.88	1.56
intermediate precision	39.38	2.63

^a Average of three concentrations 54.688, 218.750, 291.667 ng per spot ($n=3$)

Sensitivity

The sensitivity of measurement was estimated as the limits of quantification (LOQ) and detection (LOD), which were expressed based on the standard deviation of the intercept and the slope of the linearity plot as described in the related ICH guideline. The LOD and LOQ are calculated by exploiting the equations $3.3 \alpha/b$ and $10 \alpha/b$, where α is the standard deviation of the intercept and b is the slope of the corresponding calibration plot. Accordingly, the values of LOD and LOQ for sunitinib malate were found to be 23.26 and 70.50 ng per spot respectively, as it is displayed in Table IV below.

TABLE IV - LOD and LOQ values

Slope	Standard deviation of y-intercept	LOD (ng spot ⁻¹)	LOQ (ng spot ⁻¹)
4.019	28.33	23.26	70.50

Accuracy

The accuracy of the method was evaluated by analyzing samples of sunitinib malate with triplicate spotting at two levels of concentration, namely 72.92 and 291.67 ng per spot. After the development of the plate, percent recoveries were calculated from the slope and intercept of the calibration plot was achieved from the linearity study. The results obtained were compared with the expected results. As it can be seen in Table IV, the percent recoveries are within the acceptable range of 90–110%, which confirms the accuracy of the developed method, data for recoveries is presented below (Table V).

TABLE V - Recovery data for sunitinib malate

Real Concentration (ng spot ⁻¹)	Found concentration (ng spot ⁻¹)	% Recovery
72.92	68.20	93.53
291.67	294.25	100.88

Robustness

According to its definition, robustness is “a measure for the susceptibility of a method to small changes that might occur during routine analysis” (Komsta, Waksmundzka-Hajnos, Sherma, 2013). Hence, in this work, the effects of applying slight changes in the time intervals between chromatography and scanning, as well as the amount of mobile phase from 16 mL to 20 mL, were studied. The standard deviation of peak areas was calculated for each parameter at three different concentration levels of 54.69, 218.75, 291.67 ng per spot while %R.S.D. was found to be less than the acceptable value of 5%, which indicates the robustness of the method.

CONCLUSION

A new HPTLC densitometric method was developed for the analysis of sunitinib malate in bulk drug and was validated in accordance with the requirements of ICH guidelines and proved to be repeatable, precise, accurate and robust. The procedure is simple, rapid and inexpensive in comparison with other analytical methods and, at the same time, reasonably viable in order to be suggested for the routine analysis of sunitinib malate.

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