

CHAPTER 9

Sunitinib Malate

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1. INTRODUCTION

Sunitinib maleate is a multitargeted tyrosine kinase inhibitor that inhibits tumor cell proliferation and angiogenesis. It is approved for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST) [1–4]. Its activity in other tumor types (such as hepatocellular carcinoma or non-small cell lung cancer) is currently being investigated in numerous clinical trials (see www.clinicaltrials.gov). Sunitinib is given orally, once daily as a 50-mg capsule over 4 weeks, followed by a 2-week rest period, in repeated 6-week treatment cycles. At this dose, the most frequently observed adverse effects are fatigue, hypertension, diarrhea, stomatitis, and hand–foot syndrome [4].

Following oral administration, sunitinib is slowly absorbed from the gastrointestinal tract, reaching maximum plasma concentrations after about 6–12 h. It is primarily metabolized by CYP 3A4 to its active *N*-desethyl metabolite (SU12662) and is subject to presystemic metabolism by this enzyme [5]. In a mass balance study in humans with ¹⁴C-labeled sunitinib, 61% of the radioactive dose was recovered in feces and 16% in urine. In plasma samples, sunitinib and SU12662 accounted for 71% and 20.5% of the total radioactivity, respectively [6].

Approximately 95% (90%) of sunitinib (SU12662) is bound to plasma proteins. Because of the long terminal half-lives of sunitinib and SU12662

(40–60h, and 80–110h, respectively), steady-state concentrations are not achieved until 2 weeks of continuously daily dosing [2].

1.1. Nomenclature

1.1.1. Systematical chemical names

- *N*-[2-(Diethylamino)ethyl]-5-[(*Z*)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide hydrogen (2*S*)-2-hydroxybutanedioate [7]
- *N*-[2-(Diethylamino)ethyl]-5-[(*Z*)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide [7,8]
- 5-[5-Fluoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide [8]
- (2*S*)-2-Hydroxybutanedioic acid-*N*-[2-(diethylamino)ethyl]-5-[(*Z*)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide (1:1) [9]

1.1.2. Nonproprietary names

Sunitinib maleate

1.1.3. Proprietary names

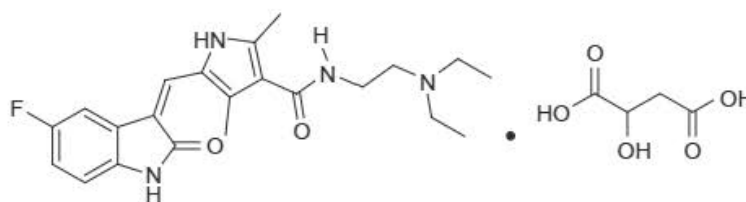
Sutent[®], Pfizer Inc., SU11248[®] [7–11]

1.2. Formulae

1.2.1. Empirical formula, molecular weight, and CAS number

Sunitinib maleate	C ₂₆ H ₃₃ FN ₄ O ₇	532.56	(341031-54-7) [7–9]
Sunitinib base	C ₂₂ H ₂₇ FN ₄ O ₂	398.47	(557795-19-4) [7–9]

1.2.2. Structural formula [7–9]



Sunitinib malate

1.2.3. Smiles

CCN(CC)CCNC(=O)C1=C(C)NC(\C=C2/C(=O)NC3=C2C=C(F)C=C3)=C1C.O=C(O)CC(O)C(=O)O

1.3. Elemental analysis

Base: C 66.31%, H 6.83%, F 4.77%, N 14.89% [7]

Malate salt: C 58.62%, H 6.22%, F 3.54%, N 10.47%

1.4. Physical properties

1.4.1. Appearance

Malate salt: yellow powder

Base: orange solid [8]

1.4.2. Solubility

Malate salt: Soluble in dimethyl sulfoxide at 40mg/mL and very poorly soluble in ethanol and water

1.4.3. Melting point

Malate salt: 194–200°C

Base: 189–191°C

1.4.4. Stability

Sunitinib malate is a photosensitive substance [12].

1.4.5. Dissociation constant

pK_a of base: 9.30 [13,14].

1.4.6. Partition coefficient (P)

For the water–octanol system: $\log P=2.5$ [15]

1.5. Uses and applications

Sunitinib is an oral multitargeted tyrosine kinase inhibitor with antiangiogenic and antitumor activities attributable to the inhibition of several related tyrosine kinase receptors, including vascular endothelial growth factor receptors (VEGFRs) types 1 and 2 (FLT1 and FLK1/KDR), platelet-derived growth factor receptors (PDGFR- α and PDGFR- β), stem cell factor receptor (c-KIT), and Fms-related tyrosine-3 receptor (FLT3), which are implicated in tumor proliferation, angiogenesis, and metastasis [1,4,5,16,17].

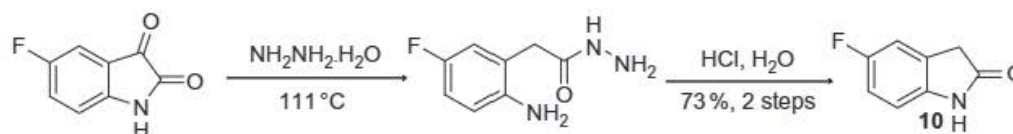
2. METHODS OF PREPARATION

2.1. Synthesis of sunitinib **1** [18–21]

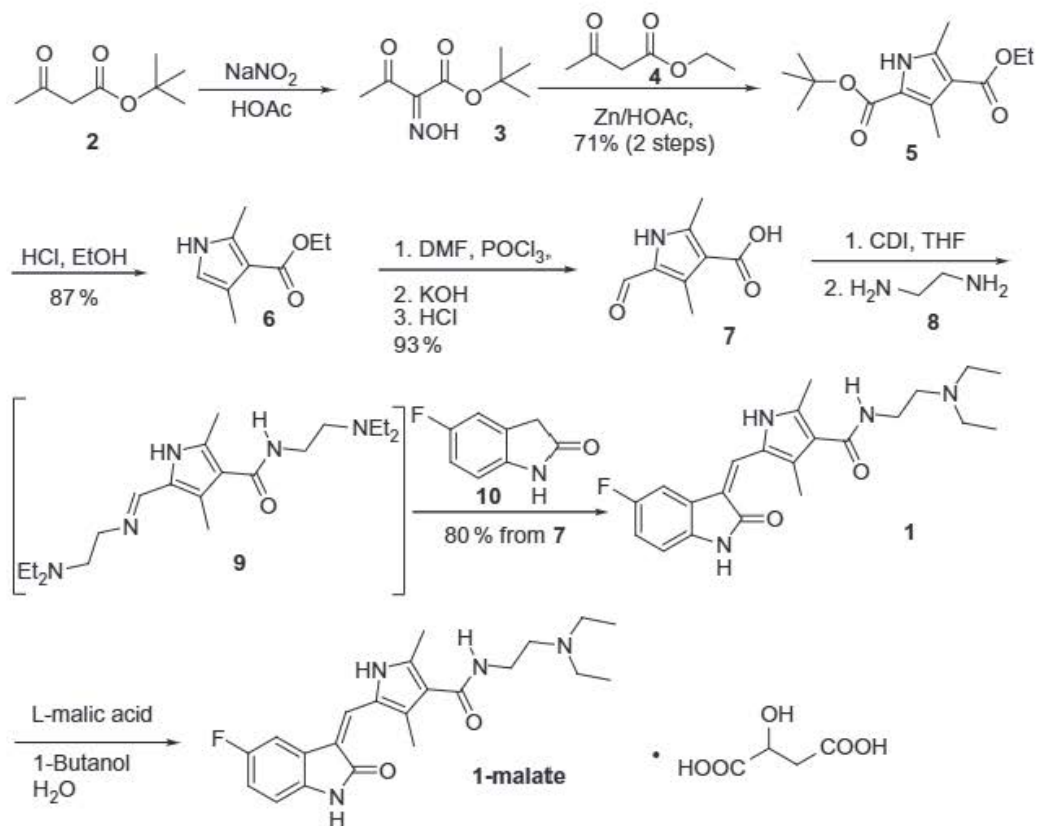
The discovery route to sunitinib **1**, as described by Sun and coworkers, commenced with a two-step synthesis of 5-fluorooxindole **10** starting from the corresponding 5-fluoroisatin [22,23]. Heating a neat mixture of 5-fluoroisatin and hydrazine hydrated to 110°C effected a Wolff–Kishner reduction and ring opening to give acyl hydrazine. The crude material was subjected to an intramolecular acylation reaction by exposure to aqueous HCl at room temperature to afford the oxindole **10** in 73% yield over two steps (Scheme 9.1).

The requisite pyrrole subunit was prepared using the Knorr pyrrole synthesis (Scheme 9.2) [23,24]. *tert*-Butyl acetoacetate **2** was reacted with sodium nitrite in acetic acid to furnish oxime **3**. Exposure of this intermediate to ethyl acetoacetate **4** under reductive cyclization conditions using zinc and acetic acid afforded the tetra-substituted pyrrole **5** in 65% yield over two steps. Selective hydrolysis of the *tert*-butyl ester, followed by decarboxylation, was accomplished by stirring **5** in HCl and ethanol to provide intermediate **6** in 87% yield. The unsubstituted position of pyrrole **6** was then formulated in quantitative yield by treatment with Vilsmeier reagent generated from DMF and POCl₃ in dichloromethane. Hydrolysis of the ethyl ester using aqueous potassium hydroxide in methanol provided the key intermediate **17** in 94% yield, thus setting the stage for the introduction of the amide side chain and the final coupling reaction with oxindole **10**. The CDI-mediated amidation of carboxylic acid **7** with diamine **8** afforded the desired amide **9**. With sufficient quantities of **9** in hand, the synthesis could be completed via condensation of **9** with 5-fluorooxindole (**10**) to furnish the sunitinib free base **1** in 88% yield.

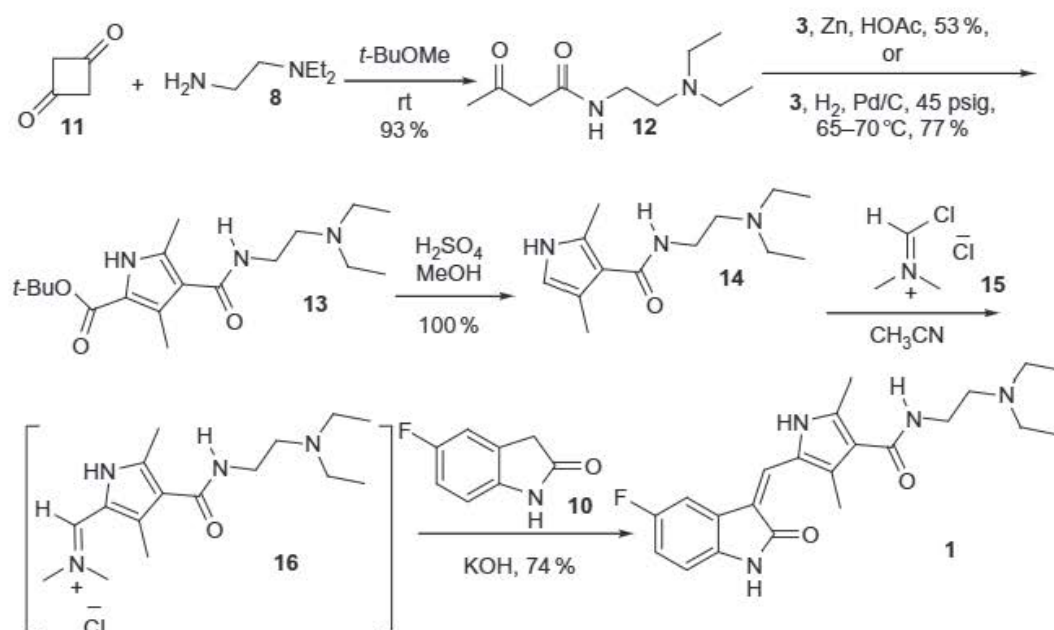
Sunitinib was also synthesized using the route described below (Scheme 9.3). Treatment of diketene with *N,N*-diethylethylenediamine in *tert*-butyl methyl ether furnished α -ketoamide **12** in excellent yield [25]. Oxime **3**, derived from *tert*-butyl acetoacetate, was treated with amide **12** in the presence of zinc and acetic acid according to the classic Knorr pyrrole formation conditions, which led to pyrrole **14**. The α -free pyrrole thus obtained **14** was treated with chloromethylene dimethylammonium



SCHEME 9.1 Synthesis of 5-fluorooxindole **10**.



SCHEME 9.2 Synthesis of sunitinib malate **1-malate** via condensation of amide **9** and 5-fluorooxindole **10**.



SCHEME 9.3 Synthesis of sunitinib free base **1** from Vilsmeier adduct **16** and 5-fluorooxindole **10**.

chloride **15** in acetonitrile to form the Vilsmeier adduct **16** *in situ* [26]. Addition of 5-fluorooxindole (**10**) and KOH to the reaction mixture at this stage afforded the desired product **1**.

3. PHYSICAL PROPERTIES

3.1. Spectroscopy

3.1.1. Ultraviolet spectroscopy

The ultraviolet/visible (UV/VIS)-absorption spectrum of sunitinib was recorded, and the wavelength of the maximum absorption peak (λ_{max}) was noted. The absorption spectrum of sunitinib in ethanol was scanned from 200 to 600 nm, using a UV/VIS spectrometer (Varian Cary 50 spectrophotometer). As shown in Fig. 9.1, the λ_{max} of sunitinib is located at 430 nm.

3.1.2. Vibrational spectroscopy

3.1.2.1. Infrared absorption spectroscopy of sunitinib base The principal peaks in the IR spectrum (NaBr pellet sampling) of sunitinib base were reported at 3298, 3230, 2968, 1676, 1627, 1590, 1544, 1498, and 1334 cm^{-1} [18].

3.1.2.2. Infrared absorption spectroscopy of sunitinib malate The infrared absorption spectrum of sunitinib is shown in Fig. 9.2 and was obtained on a Shimadzu infrared spectrophotometer using a NaCl plate. The principal peaks were observed at 3350, 2943, 2831, 1650, and 1031 cm^{-1} .

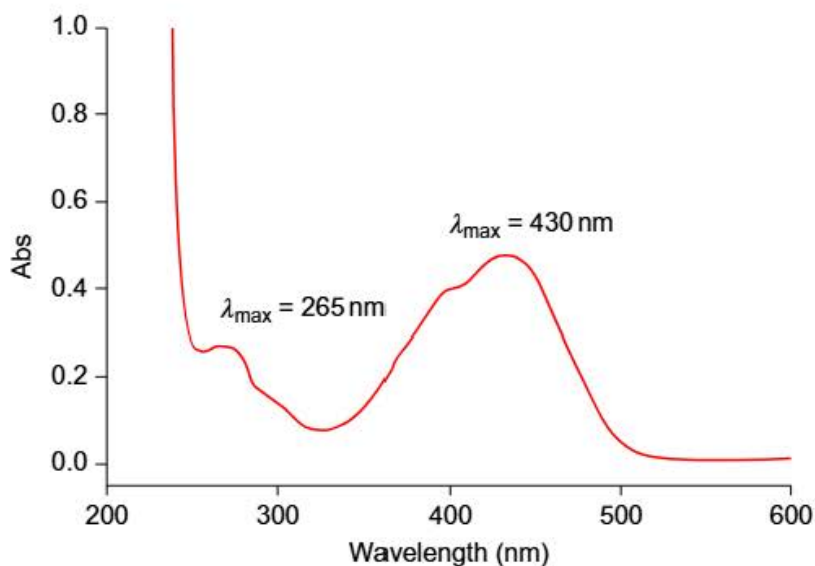


FIGURE 9.1 UV spectra of sunitinib malate (4.0 μM solution in ethanol).

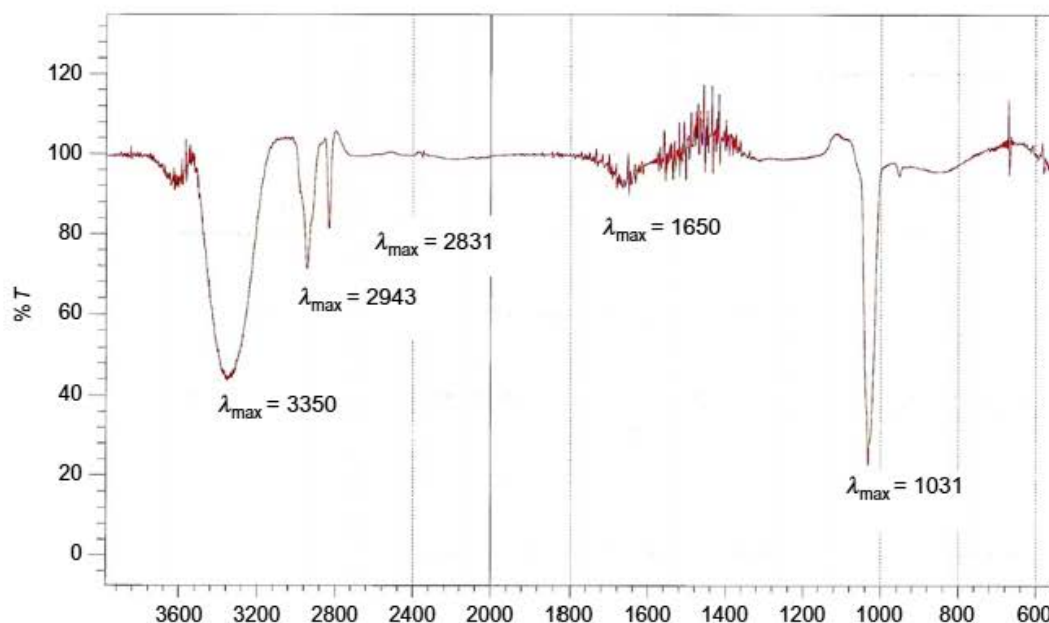


FIGURE 9.2 Infrared spectroscopy of sunitinib malate.

TABLE 9.1 Infrared spectroscopic data for sunitinib malate in NaCl plate

Entry	Bond	Absorption peaks ($\lambda_{\max}$ cm ⁻¹)	Appearance
1	C—F	1031	Strong
2	—NH—C=O	1650	Normal
3	C—H (alkyl)	2831	Strong
4	HC=CH (aryl)	2943	Strong
5	O—H (acid), N—H	3350	Broad

Assignments for the major absorption bands are provided in [Table 9.1](#). Reported principal peaks were at 3298, 3230, 2968, 1676, 1627, 1590, 1544, 1498, 1334 cm⁻¹ [18].

3.1.3. Nuclear magnetic resonance spectrometry

¹H and ¹³C nuclear magnetic resonance (NMR) spectra of sunitinib were obtained with a Bruker 500MHz NMR spectrometer. Chemical shifts were expressed in parts per million (ppm) ([Table 9.2](#)) with respect to the tetramethylsilane reference signal for ¹H and ¹³C NMR ([Figs. 9.3 and 9.4](#), respectively).

3.1.3.1. ¹H NMR spectrum ¹H NMR (500MHz, DMSO-*d*₆): δ 13.73 (s, 1H, NH), 10.93 (s, 1H, NH), 10.80–10.70 (br, 1H, —OH, maleate), 7.77 (d, 1H, *J*=8.0Hz), 7.73 (s, 1H, olefinic H), 7.72 (d, 1H, *J*=8.0Hz), 6.93 (d, 1H, *J*=8.0

TABLE 9.2 Comparative study of ¹H NMR spectra for sunitinib malate with literature

Entry	Chemical shift ^a (sunitinib malate) (500MHz, DMSO- <i>d</i> ₆)	Chemical shift ^b (400MHz, CDCl ₃)	Chemical shift ^c (300MHz, CDCl ₃)
1	13.73 (s, 1H, NH)	–	13.31 (s, 1H, NH)
2	10.93 (s, 1H, NH)	–	8.90 (s, 1H, NH)
3	10.80–10.70 (br, 1H, —OH, malate)	–	–
4	7.77 (d, 1H, <i>J</i> =8.0Hz)	7.75 (dd, <i>J</i> =9.4, 2.5Hz, 1H),	7.14 (dd, 1H, <i>J</i> =8.8, 2.2Hz)
5	7.73 (s, 1H, olefinic H)	7.71 (s, 1H)	7.22 (s, 1H, olefinic H)
6	7.72 (d, 1H, <i>J</i> =8.0Hz)	7.43 (t, <i>J</i> =5.6Hz, 1H)	6.85 (td, 1H, <i>J</i> =8.8 2.2Hz)
7	6.93 (d, 1H, <i>J</i> =8.0Hz)	6.92 (td, <i>J</i> =9.1, 2.5Hz, 1H)	6.78 (dd, 1H, <i>J</i> =8.1, 4.4Hz)
8	6.87 (d, 1H, <i>J</i> =8.0Hz)	6.84 (dd, <i>J</i> =8.5, 4.6Hz, 1H)	6.72 (t, 1H, <i>J</i> =4.4Hz)
9	4.01 (s, 1H, malate)	–	–
10	3.48 (br, 2H)	3.41–3.36 (m, 2H)	3.54 (q, 2H, <i>J</i> =5.2Hz)
11	2.96 (s, 6H)	2.65–2.58 (m, 6H)	2.71 (t, 2H, <i>J</i> =5.9Hz), 2.64 (q, 4H, <i>J</i> =6.6Hz)
12	2.56 (q, 1H, malate)		
13	2.34 (q, 1H, malate)		
14	2.51 (s, 3H)	2.47 (s, 3H)	2.55 (s, 3H)
15	2.44 (s, 3H)	2.43 (s, 3H)	2.31 (s, 3H)
14	1.14 (t, <i>J</i> =7.3Hz, 6H)	1.07 (t, <i>J</i> =7.1Hz, 6H)	1.05 (t, 6H, <i>J</i> =7.4Hz)

^a ¹H NMR was taken in DMSO-*d*₆.^b Reference [18].^c Reference [20].

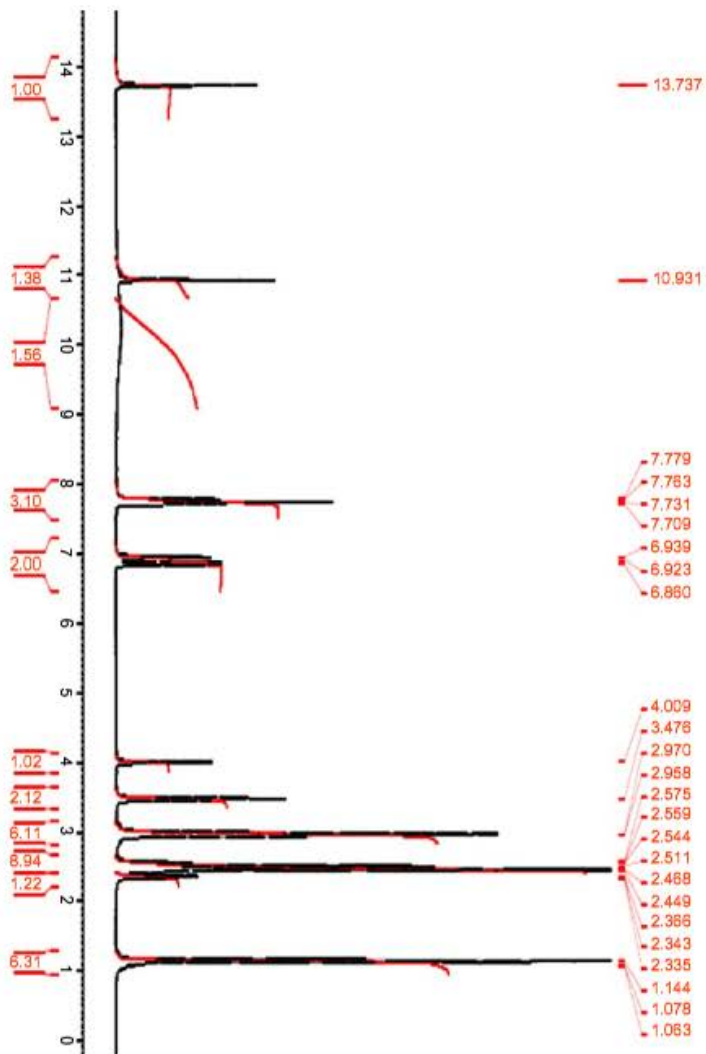


FIGURE 9.3 ¹H NMR spectra of sunitinib malate in DMSO-*d*₆.

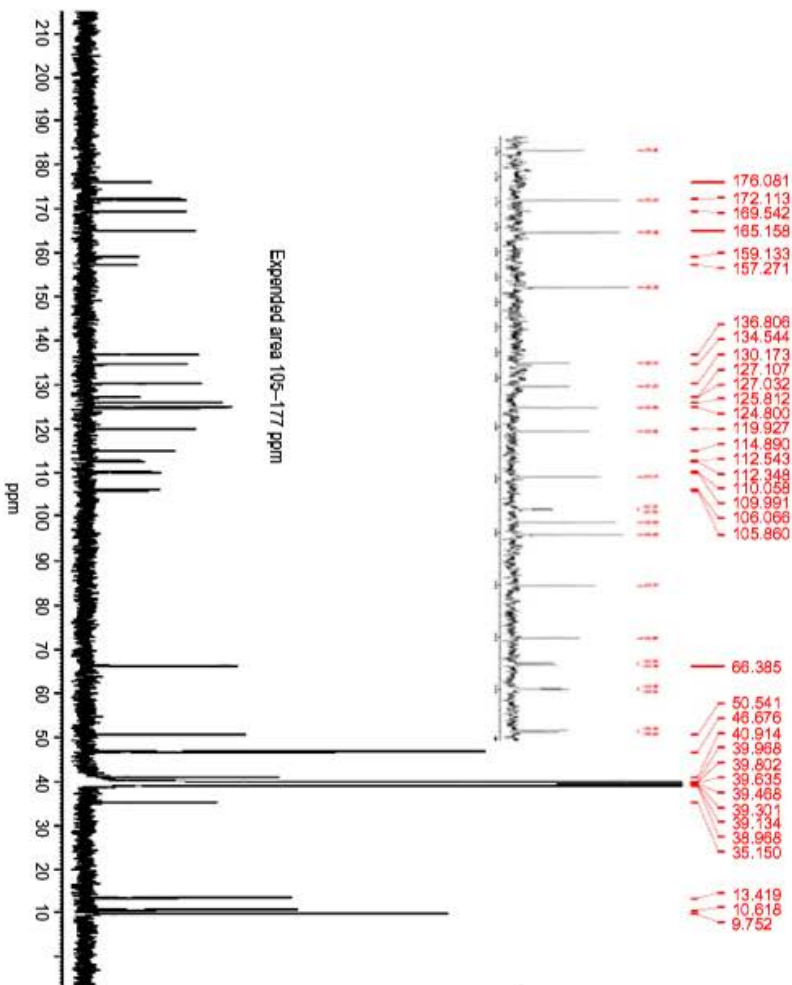


FIGURE 9.4 ¹³C NMR spectra of sunitinib malate in DMSO-*d*₆.

Hz), 6.87 (d, 1H, $J=8.0$ Hz), 4.01 (s, 1H, malate), 3.48 (br, 2H), 2.96 (s, 6H), 2.56 (q, 1H, malate), 2.34 (t, 1H, malate), 2.51 (br, 2H), 1.14 (s, 6H)ppm.

3.1.3.2. ^{13}C NMR spectrum Unreported ^{13}C NMR data of sunitinib malate are as follows: ^{13}C NMR (125MHz, $\text{DMSO}-d_6$): δ 176.08 (malate), 172.11 (malate), 169.54, 165.16, 159.13, 157.27, 136.80, 134.54, 130.17, 127.10, 127.03, 125.81, 124.80, 119.93, 114.89, 112.54, 112.35, 110.06, 109.99, 106.07, 105.86, 66.38 (malate), 50.54, 46.68, 40.91 (malate), 35.16, 13.42, 10.62, and 9.75ppm.

3.2. Mass spectrometry

The mass spectral analysis of sunitinib was carried out with an Agilent 6320 Ion Trap LC/MS system by infusion of 2 μg of sunitinib solution in 1:1 acetonitrile/water without a column. Smart fragmentor and automatic optimization were performed to obtain the spectra and cause fragmentation. Source parameters were as follows: temperature was 350°C, gas flow was 12L/min, and the nebulizer was set at 60psi. Figure 9.5 shows the mass spectrum for the parent compound (m/z 398); Scheme 9.4 and Figs. 9.6–9.8 show the detailed mass fragmentation pattern interpretation of the drug substance. The reported m/z was 399.2204 ($\text{M}+\text{H}^+$), $\text{C}_{22}\text{H}_{27}\text{FN}_4\text{O}_2+\text{H}$ (theoretical 399.2196) [27].

Sunitinib malate shows 398.7 (m/z) as a metastable ion peak (Fig. 9.5).

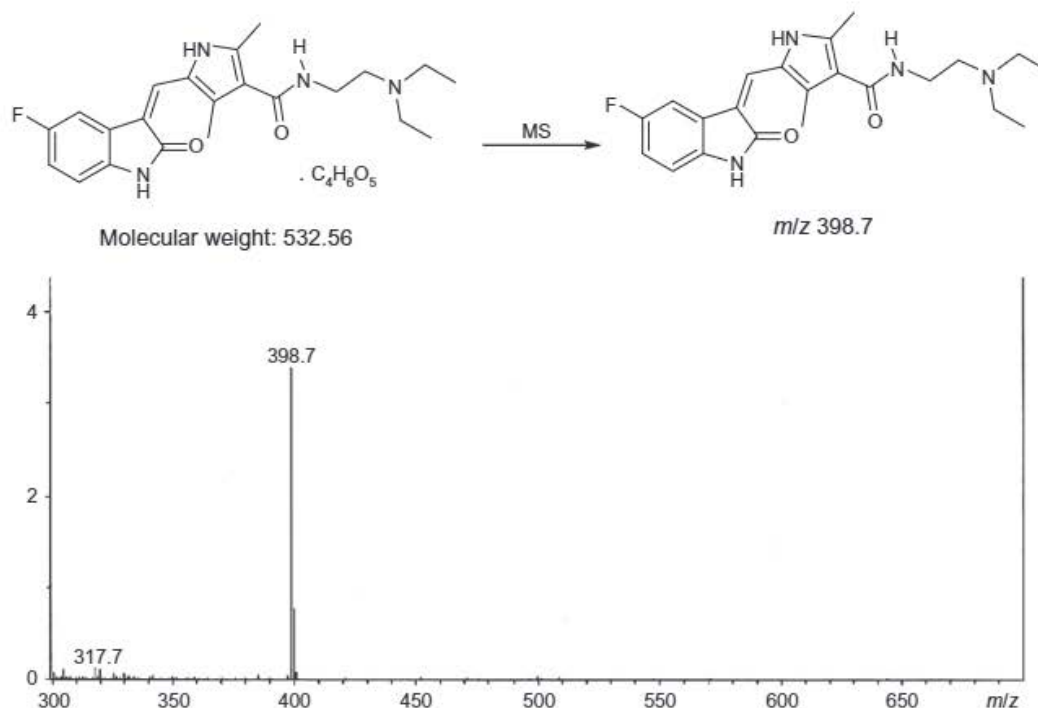
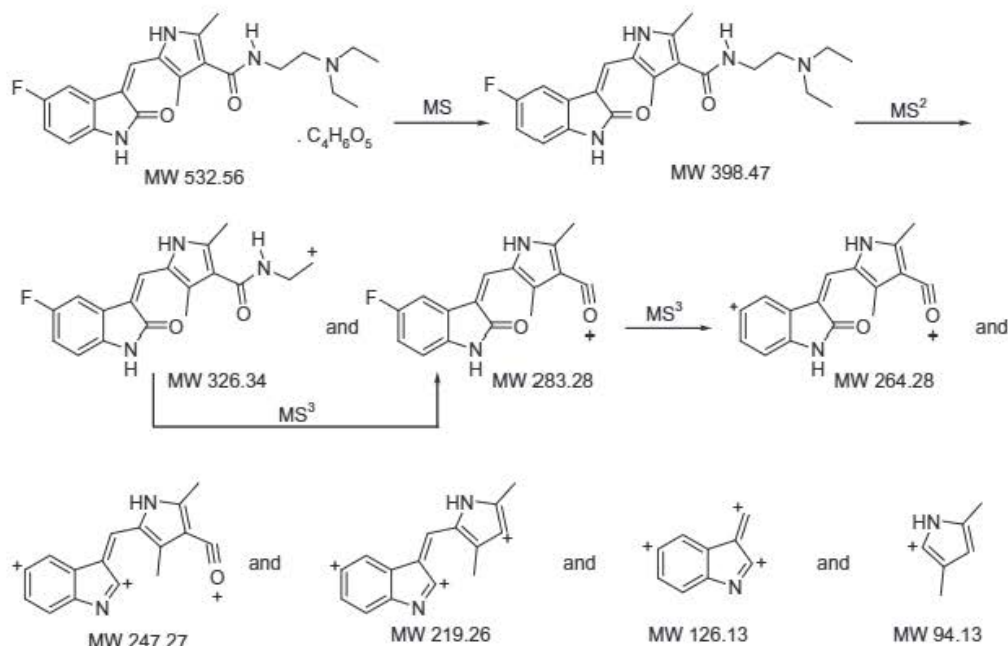


FIGURE 9.5 MS spectra for fragment sunitinib malate molecular weight 532.56 in positive mode gave m/z 398.47 [$\text{M-malate}]^+$.

**SCHEME 9.4** Fragmentation of sunitinib malate.

3.2.1. Fragmentation pattern of sunitinib

MS^2 scan of the metastable ion peak gives two fragments at 398.7 (m/z , by loss of diethylamine) and 282.7 (m/z , by loss of N,N -diethylethylene diamine), respectively (see Fig. 9.6).

MS^3 scan of the fragment at 398.7 (m/z) also gave 282.7 (m/z , by loss of ethylamine) (see Fig. 9.7).

MS^3 scan of the 284.7 (m/z) gave the number of fragments majoring 264.7 (m/z), 247.8 (m/z), 217.8 (m/z), 128.4 (m/z), and 94.6 (m/z), respectively (Fig. 9.8).

4. METHODS OF ANALYSIS

4.1. Chromatographic methods

4.1.1. High-performance liquid chromatography (ultraviolet detection)

There are a few high-performance liquid chromatography (ultraviolet detection) (HPLC/UV) methods published to determine sunitinib, either as a single component or simultaneously with its metabolite. In the method developed by Blanchet *et al.* [28], after a liquid–liquid extraction with ethyl acetate, sunitinib and ranitidine (the internal standard (IS)) are separated on cyanopropyl column using a simple binary mobile phase of ammonium acetate buffer (20mM, pH 6.8):acetonitrile (55:45, v/v). Samples were eluted isocratically at a flow rate of 1 mL/min throughout the 10-min run. A dual wavelength mode was used, with ranitidine

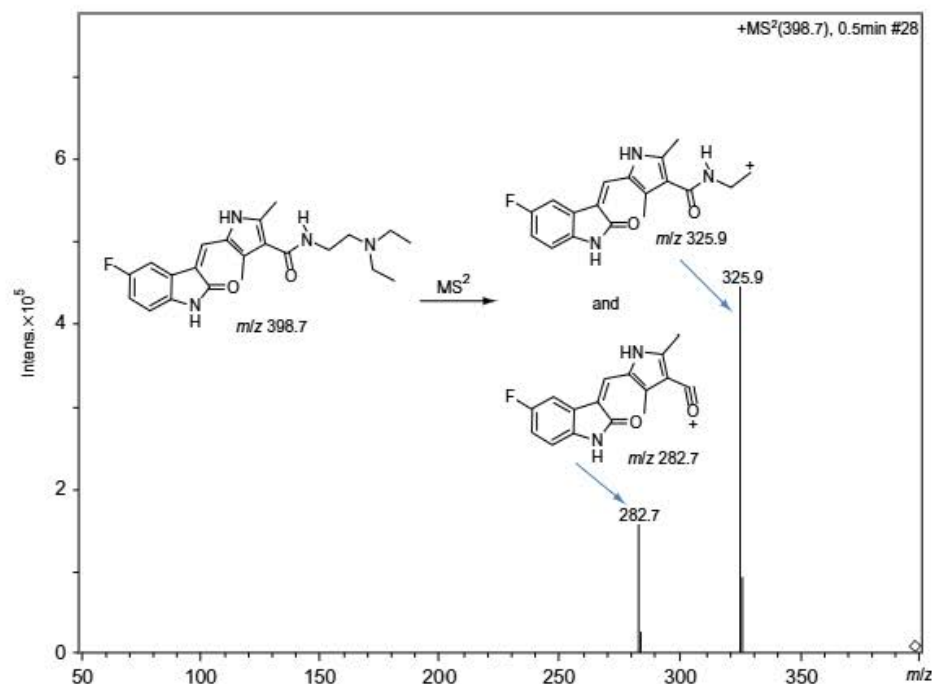


FIGURE 9.6 MS² spectra for fragment 398.7 (*m/z*) in positive mode gave 325.9 (*m/z*) and 282.7 (*m/z*).

monitored at 255nm and sunitinib at 431nm. The calibration was linear over the range of 20–200ng/mL. Inter- and intraday coefficients of variation were less than 7%. This method is sensitive, accurate, and selective. It has been successfully implemented to monitor trough sunitinib concentrations in plasma samples ($n=39$) from 14 unselected cancer patients treated with the recommended once-daily dose of 50mg or less. It has been concluded that this method can be used in routine clinical practice to monitor plasma sunitinib concentrations in cancer patients treated with once-daily administration.

Etienne-Grimaldi *et al.* [12] developed an original, simple, HPLC assay with UV detection, allowing the simultaneous measurement of sunitinib and SU12662 to be performed in plasma after a single organic extraction. This simple and robust analytical assay could be helpful in the setting of pharmacokinetically based dose adaptation for sunitinib. In addition, a stability study of sunitinib and SU12662 in different light exposure conditions was presented. HPLC analysis was performed on an ODS column, and UV detection was monitored at 369nm (run time: 15min). The IS was vandetanib. The mobile phase was composed of 60% ammonium formate 20mM (pH 3.25, adjusted with formic acid) and 40% acetonitrile. The flow rate was 0.8mL/min. This assay was selective and sensitive enough (limit of detection: ~1ng/mL) to quantify minimal concentrations at steady state (C_{ss} min) of sunitinib and SU12662 in treated patients.

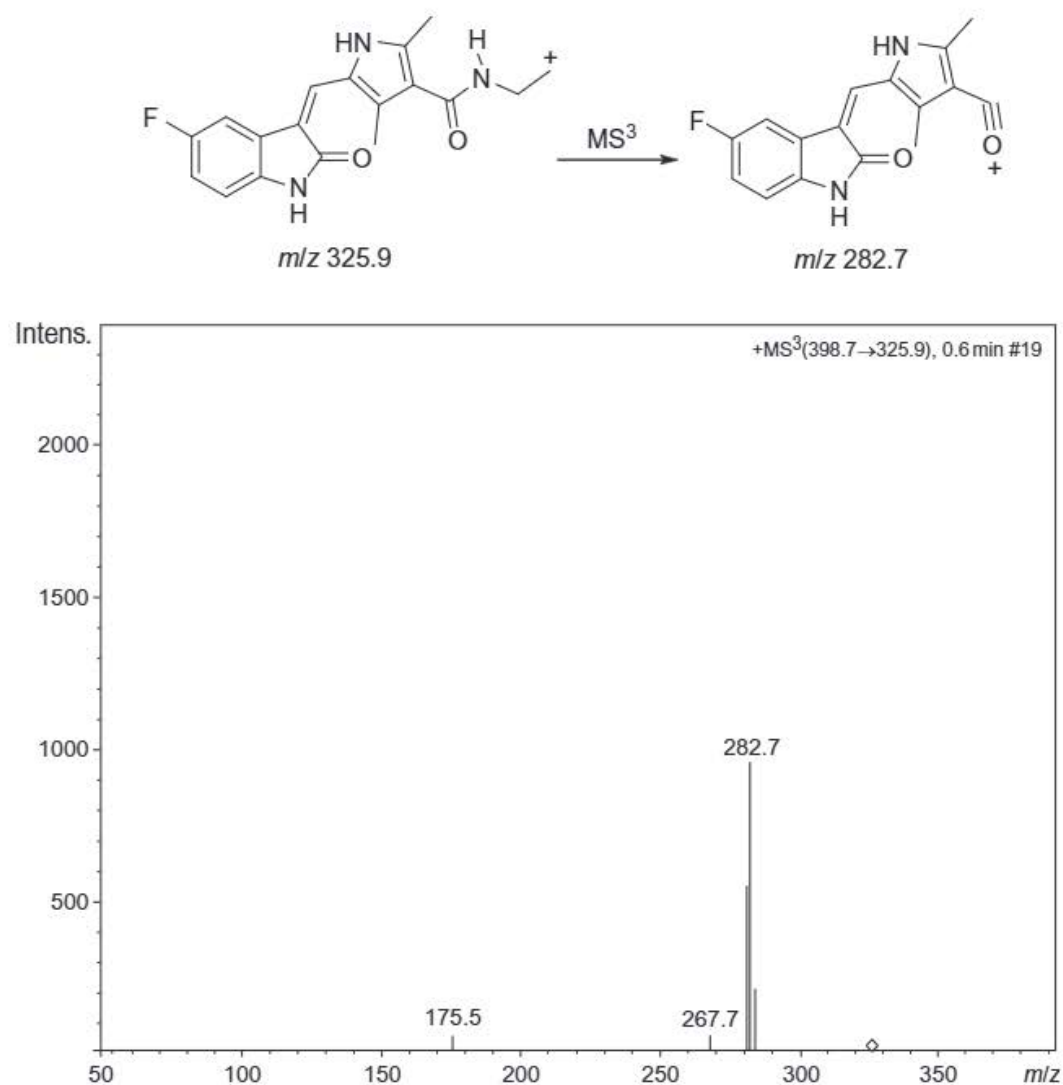


FIGURE 9.7 MS^3 spectra for fragment 398.7 (m/z) in positive mode gave 282.7 (m/z).

4.1.2. High-performance liquid chromatography/mass spectrometry

Several coupled high-performance liquid chromatography/mass spectrometry (LC-MS/MS) methods have been reported to determine sunitinib, either as a single drug or in combination with other tyrosine kinase inhibitors and/or its metabolite SU12662 in plasma [29–34].

Minkin *et al.* [29] developed and validated an assay using LC-MS/MS for the determination of sunitinib in human plasma. The assay's lower limit of quantitation (LLOQ) is 0.2 ng/mL, with a simple sample preparation procedure and a rapid chromatographic run time of 3 min. Sample preparation involved a liquid–liquid extraction by the addition of 0.2 mL of plasma with 4.0 mL *tert*-butyl-methyl ether extraction solution containing 25 ng/mL of the IS clozapine. Separation of compounds was achieved on a C18 (50 × 2.1 mm i.d., 3.5 μm) analytical column using a mobile phase

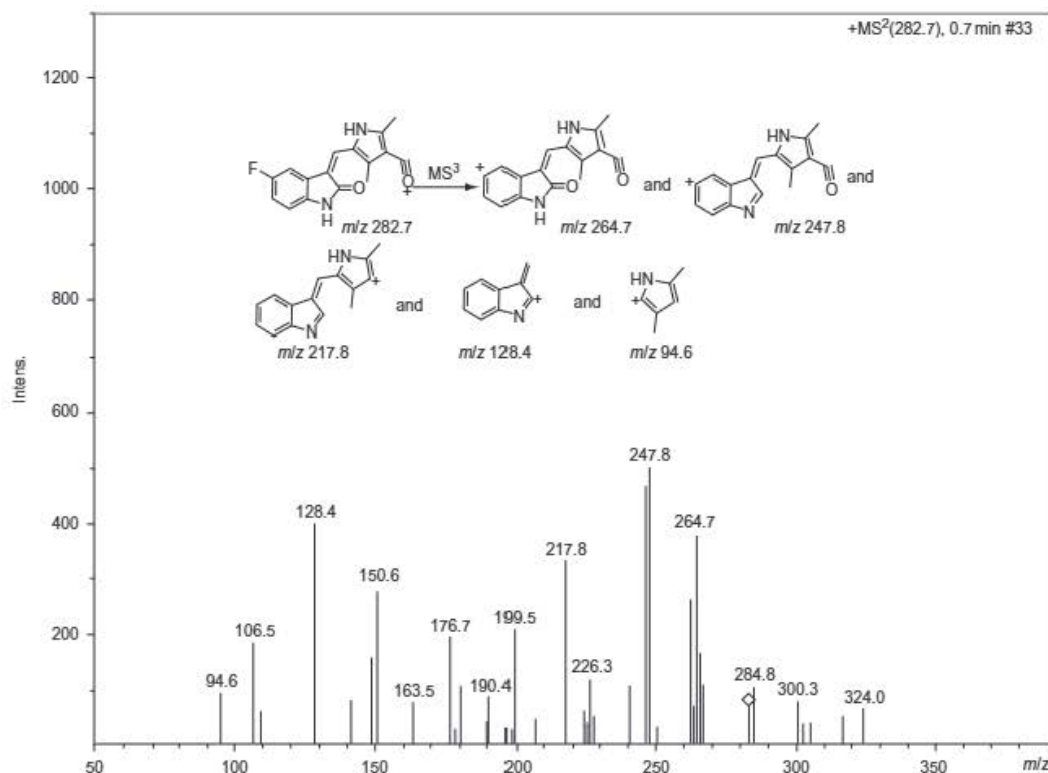


FIGURE 9.8 MS³ spectra for fragment 282.7 m/z in positive mode.

consisting of acetonitrile/water (65:35, v/v) containing 0.1% formic acid and isocratic flow at 0.15 mL/min for 3 min. The analytes were monitored by tandem-mass spectrometry with electrospray positive ionization. The mass spectrum of sunitinib showed a protonated molecular ion $[MH]^+$ at m/z 399.0. The major fragment observed was at m/z 283.0, which was selected for subsequent monitoring in the third quadrupole. The mass spectrum of the IS, clozapine, showed a $[MH]^+$ at m/z 327.0, and the high collision energy gave a major product ion at m/z 270.0. Linear calibration curves in human plasma were generated over the range of 0.2–500 ng/mL with values for the coefficient of determination of exceeding 0.9950. Within- and between-day precision and accuracy were less than or equal to 10%. The method was applied to the quantitation of sunitinib in plasma samples from a patient receiving daily oral therapy with sunitinib.

A fast, sensitive, universal, and accurate LC-MS/MS method for the determination of four different tyrosine kinase inhibitors (TKIs) from biological materials was developed by Honeywell *et al.* [30]. Utilizing a simple protein precipitation with acetonitrile, a 20- μ L sample volume of biological matrixes can be extracted at 4°C with minimal effort. After centrifugation, the sample extract is introduced directly onto the LC-MS/MS system without further cleanup and assayed across a linear

range of 1–4000 ng/mL. Chromatography was performed using a Dionex Ultimate 3000 with a Phenomenex prodigy ODS3 (2.0×100 mm, 3 μm) column and eluted at 200 μL/min with a tertiary mobile phase consisting of 20 mM ammonium acetate/acetonitrile/methanol (2.5:6.7:8.3%). Injection volumes varied from 0.1 to 1 μL depending on the concentration of the drug observed. Samples were observed to be stable for a maximum of 48 h after extraction when kept at 4 °C. Detection was performed using a turbo-spray ionization source and mass spectrometric positive multiple reaction monitoring (+MRM) mode for gefitinib (447.1 and 127.9 *m/z*), erlotinib (393.9 and 278.2 *m/z*), sunitinib (399.1 and 283.1 *m/z*), and sorafenib (465.0 and 251.9 *m/z*) at an ion voltage of +3500 V. The accuracy, precision, and limit of quantification (LOQ) from cell culture medium were as follows: gefitinib: 100.2±3.8%, 11.2 nM; erlotinib: 101.6±3.7%, 12.7 nM; sunitinib: 100.8±4.3%, 12.6 nM; sorafenib: 93.9±3.0%, 10.8 nM, respectively. This was reproducible for plasma, whole blood, and serum. The method was observed to be linear between the LOQ and 4000 ng/mL for each analyte. Effectiveness of the method was illustrated with the analysis of samples from a cellular accumulation investigation and determination of steady-state concentrations in clinically treated patients.

Haouala *et al.* [31] developed a liquid chromatography-tandem-mass spectrometry method (LC-MS/MS), requiring 100 μL of plasma for the simultaneous determination of the six major inhibitors of TKI kinases imatinib, nilotinib, dasatinib, sunitinib, sorafenib, and lapatinib. Plasma is purified by protein precipitation, and the supernatant is diluted in ammonium formate 20 mM (pH 4.0). Reverse-phase chromatographic separation of TKIs is obtained using a gradient elution of 20 mM ammonium formate, pH 2.2 and acetonitrile containing 1% formic acid, followed by rinsing and re-equilibration to the initial solvent composition up to 20 min. Imatinib-*d*₈ was used as the IS.

Analyte quantification, using matrix-matched calibration samples, is performed by electrospray ionization (ESI)-triple quadrupole mass spectrometry by selected reaction monitoring detection using the positive mode. The method was validated according to FDA recommendations, including assessment of extraction yield, matrix effects variability (<9.6%), overall process efficiency (87.1–104.2%), as well as TKIs short- and long-term stability in plasma. The method is precise (interday CV%: 1.3–9.4%), accurate (−9.2 to +9.9%), and sensitive (lower limits of quantification comprised between 1 and 10 ng/mL). This is the first broad-range LC-MS/MS assay covering the major currently in-use TKIs. It is an improvement over previous methods in terms of convenience (a single extraction procedure for six major TKIs, reducing significantly the analytical time), sensitivity, selectivity, and throughput. It may contribute to filling the current knowledge gaps in the pharmacokinetics/pharmacodynamics

relationships of the latest TKIs developed after imatinib and better define their therapeutic ranges in different patient populations in order to evaluate whether a systematic TDM-guided dose adjustment of these anticancer drugs could contribute to minimize the risk of major adverse reactions and to increase the probability of efficient, long-lasting, therapeutic response.

A high-performance LC-MS/MS method was developed and validated by Haznedar [32] for measurement of sunitinib and SU12662 concentrations in rat plasma samples. The samples were prepared for analysis either by an ethyl acetate extraction (used in only a few initial studies) or after protein precipitation (in test tubes or 96-well plates). The assay used either propranolol or [2H10]-sunitinib as an IS. The concentration range of either 1–2000 or 0.1–200 ng/mL was validated using a Keystone BetaBasic-18 column (4.6×9×100 mm, 5 L) for LC-MS/MS analysis (Shimadzu Series 10 ADVP HPLC system and Perkin-Elmer Sciex API-3000 triple quadrupole mass spectrometer). The gradient mobile phases were 10 mM ammonium acetate in methanol/water (10:90) with 0.1% formic acid (A) and 10 mM ammonium acetate in methanol/water (90:10) with 0.1% formic acid (B). The flow rate was 0.8 mL/min, which was reduced to 0.3 mL/min before introduction into the mass spectrometer. The mass spectrometer was operated using ionization and scan modes of positive ion electrospray and MRM, respectively. Each analyte was detected by tandem MS by monitoring specific molecular ion fragment ion transitions: m/z 399.1 and 325.9 for sunitinib, 371.1 and 283.1 for SU12662, and 360.1 and 116.0 for propranolol. Calibration curves were constructed by plotting the analyte/IS peak area ratios against the analyte concentrations. A weighted linear regression (1/C²) was used to calculate the concentrations of sunitinib and SU12662 in the study and QC samples. This assay method demonstrated acceptable selectivity/specificity, linearity, and intra- and interassay precision and accuracy. The blank matrix also showed no significant interference or carryover on the LC-MS/MS system. The LLOQ was 0.1 ng/mL for both sunitinib and SU12662.

Alternatively, a Symmetry Shield C8 column (2.1×50 mm, 3.5 L) was used to perform the chromatographic separation. The mobile phase was 15 mM ammonium formate buffer solution (pH 3.25)/acetonitrile (75:25, v/v), with a flow rate of 0.35 mL/min. The mass spectrometer was operated using MRM (m/z for sunitinib and SU12662 were as described above; m/z 409.1, 325.9 for IS) and in a positive ion mode. The lower and upper limits of quantitation were 0.07 and 241 ng/mL for sunitinib and 0.07 and 220 ng/mL for SU12662. This method also showed acceptable assay specificity/selectivity, linearity, sensitivity, precision, accuracy, and stability. Radioactivity in rat and monkey blood, plasma, urine, and fecal samples was measured by liquid scintillation counting (Packard 1900 or 2100 TR Liquid Scintillation Counter). The counting efficiencies were calculated by the external standard method, using a series of quenched standards

(supplied by Packard), generating a calibration curve. The purpose of these extensive nonclinical studies was to assess pharmacokinetics and dispositional properties of sunitinib and its primary active metabolite (SU12662). Sunitinib was administered in single and repeat oral doses in mice, rats, and monkeys. Assessments were made using LC-MS/MS methods, radioactive assays, and quantitative whole body autoradiography. Sunitinib was readily absorbed with good oral bioavailability and linear kinetics at clinically-relevant doses. SU12662 plasma levels were less than those of sunitinib in mice and monkeys, but greater in rats. Sunitinib was extensively distributed with moderate-to-high systemic clearance and eliminated primarily into feces. Single- and repeat-dosing kinetics were similar. A prolonged half-life allowed once-daily dosing, enabling adequate systemic exposure with limited-to-moderate accumulation. In multiple-dose studies with cyclic dosing, drug plasma concentrations cleared from one cycle to the next. Sunitinib exhibited advantageous pharmacokinetic and dispositional properties in nonclinical species, translating into favorable properties in humans.

A LC-MS/MS-based method combined with protein precipitation, liquid-liquid extraction, and solid-phase extraction techniques was developed by Zhou *et al.* [33] for the determination of sunitinib in mouse plasma, brain tumor, and normal brain tissue, respectively. The instrument was operated under the MRM mode using ESI in the positive ion mode. Separation was achieved on a 50×2.0mm Luna 3μm C8 column (Phenomenex Inc., Torrance, CA, USA) with a pre-column of the same material. The sample solutions (10μL) were injected, and the analytes were eluted using acetonitrile/1mM ammonium acetate containing 0.1% acetic acid (28:72, v/v) at a flow rate fixed at 0.3mL/min. The isocratic separation run was completed within 3.2min at 30°C. Camptothecin was used as the IS. A good linear relationship with correlation coefficients greater than 0.99 was achieved over concentration ranges of 1.37–1000ng/mL for plasma and 4.12–1000ng/g for the normal brain and brain tumor. The LOQs for sunitinib in mouse plasma, brain tumor, and normal brain tissue are 1.37ng/mL, 4.12ng/g, and 4.12ng/g, respectively. The reproducibility of the LC-MS/MS method is reliable, with the intra- and interday precision being less than 15% and accuracy within ±15%. The established method was successfully applied to the characterization of sunitinib disposition in the brain and brain tumor as well as its systemic pharmacokinetics in a murine orthotopic glioma model.

In a thesis having the title “Pharmacokinetic/Pharmacodynamic Modeling and Simulation of Biomarker Response to Venlafaxine and Sunitinib Administration” [33], Sunitinib and SU12662 concentrations in human plasma were determined by HPLC coupled with mass spectrometry (LC-MS/MS, Applied Biosystems/MDS Sciex API5000 LC-MS/MS). This work was performed by Dr. Martina Kinzig at the Institute for Biomedical

and Pharmaceutical Research (IMBP) under the supervision of Prof. Dr. Fritz Sörgel. The method used d_5 -sunitinib as an IS which was synthesized by Elsinghorst [21]. The response from calibration standards of sunitinib and SU12662 was linear from 0.06 to 100 ng/mL. The LLOQ for plasma samples was 0.06 ng/mL for both analytes. Coefficients of variation (CV) expressing precision and relative errors (RE) expressing analytical accuracy ranged from 1.0% to 5.3% and +0.9% to +5.6% for sunitinib and from 2.9% to 3.9% and -2.2% to +3.9% for SU12662, respectively.

4.1.3. Ultra-performance liquid chromatography/mass spectrometry

Simultaneous determination of nine TKIs in plasma samples by 96-well solid-phase extraction and ultra-performance LC-MS/MS published by Bouchet *et al.* [35]. The described chromatography was performed on a Waters Acquity-UPLC[®] system with BEH C18-50×2.1 mm column, under a gradient of ammonium formate–acetonitrile. An Acquity-TQD[®] system with ESI was used for detection. Samples were prepared by solid-phase extraction (Oasis[®] MCX μ Elution), and eluate was injected in the system. Calibration curves ranged from 10 to 5000 ng/mL for imatinib, its metabolite, nilotinib, lapatinib, erlotinib and sorafenib, and from 0.1 to 200 ng/mL for dasatinib, axitinib, gefitinib, and sunitinib. Peaks of each compound (retention time from 0.76 to 2.51 min) were adequately separated. The mean relative extraction recovery was in the range of 90.3–106.5% thanks to the use of stable isotopes (imatinib- d_8 , nilotinib-M+4, dasatinib-M+6, and sorafenib-M+4) as ISs. There was no significant ion suppression observed at the respective TKI retention times. This rapid, sensitive, and specific UPLC-MS/MS method is able to perform simultaneous quantification of nine TKIs in human plasma and usable for routine TDM.

4.2. Immunoassay methods

Establishment of a homogeneous time-resolved fluorescence immunoassay method for high-throughput screening of protein TKIs was reported by Li and coworkers [36]. Specific fluorescence signals at 670 and 612 nm were measured by multifunctional microplate reader when the fluorescence was emitted through a resonance energy transfer between fluorescent materials (XL-665 and Eu^{3+}). The inhibitory activity of sunitinib, a standard PTK inhibitor, on vascular endothelia growth factor receptor 2 (VEGFR-2) kinase activity was investigated. A homogeneous time-resolved fluorescence immunoassay was established for high-throughput screening of PTK inhibitor. In this system, the concentrations of VEGFR-2, adenosine triphosphate (ATP), and polypeptide substrate were 5 ng/ μ L, 100 μ M/L, and 50 μ M/L, respectively. Sunitinib inhibited VEGFR-2 kinase activity with an IC_{50} value of 86.7 nmol/L, which was close to the values

tested using the other method. The homogeneous time-resolved fluorescence immunoassay can be easily used for high-throughput screening of PTK inhibitors.

4.3. Spectrofluorimetry

Arici *et al.* [37] described a validated spectrofluorimetric method for the determination of sunitinib malate, using a dye complexation approach. The method was developed for the indirect determination of sunitinib malate in bulk substance and in pharmaceutical preparations. The complex formed in this reaction system was regarded as the ion-association complex between the drug cation and Eosin Y. This method has the advantages of simplicity, sensitivity, and reproducibility and is useful and convenient for quality control and routine determination of drugs in pharmaceutical dosage forms where precision, time, and cost effectiveness of analytical methods are important. The dye complexation spectrofluorimetric quantification of sunitinib malate was performed by monitoring the emission of the free dye at 800nm, following its excitation at 350nm. The described method was fully validated as to the analytical parameters of accuracy, linearity, specificity, limit of detection, limit of quantification, precision, reproducibility, and robustness. The described method displayed linearity over the concentration range of 0.08–5.00 µg/mL. The limit of detection was found to be 0.041 µg/mL (7.69×10^{-8} M), and the limit of quantitation was 0.850 µg/mL (1.59×10^{-6} M). Defined parameters of the method (such as medium pH, dissolved oxygen concentration, temperature, dye concentration, incubation time, etc.) were varied systematically to check robustness. Reproducibility was tested by applying the proposed method to the assay of sunitinib malate using the same operational conditions, namely in two different laboratories at different elapsed time and using different instruments. Results obtained from lab-to-lab variations were found to be reproducible, as RSD values did not exceed 2%.

5. PHARMACOLOGY

Sunitinib malate (Sutent[®], SU11248) is an oral, multitargeted TKI that specifically inhibits vascular endothelial growth factor receptors 1, 2 and 3 (VEGFR1, -2 and -3, respectively), platelet-derived growth factor receptor alpha and beta (PDGFR- α and - β), FLT3 colony-stimulating factor receptor type 1 (CSF-1R) and the receptor encoded by the ret proto-oncogene (RET) [38,39]. Sunitinib is approved for first line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic GIST [5,40,41]. Sunitinib is metabolized by cytochrome P450 (CYP) 3A4 to in active metabolites [42].

Clinical pharmacokinetics of sunitinib show a high inter-patient variability (approximately 40%), which is largely unexplained [5]. This could result in supra- or sub-therapeutic sunitinib levels leading to toxicity or inefficacy, respectively. Since sunitinib is predominantly metabolized by CYP3A4, variability in the activity of this enzyme may explain a considerable proportion of the observed inter-patient variability in sunitinib pharmacokinetics.

It is thought that sunitinib is neither an inhibitor nor an inducer of CYP-enzymes and therefore the drug is considered not prone to drug-drug and drug-food interactions, while other TKIs (e.g. imatinib, erlotinib, gefitinib) appear to be substrates and/or inhibitors of several CYP-enzymes *in vivo* and *in vitro* [1,33,43–47]. For sunitinib, *in vivo* confirmatory studies to define an effect of sunitinib on CYP-enzymes are lacking. Moreover, recently it was shown in an *in vitro* study that sunitinib is a substrate for and an inhibitor of the transporter proteins ATP-binding cassette (ABC) ABCG2 and to some extent ABCB1, which may also lead to drug–drug interactions [48].

Mechanism of Action. Sunitinib malate is a small molecule that inhibits multiple receptor tyrosine kinases (RTKs), some of which are implicated in tumor growth, pathologic angiogenesis, and metastatic progression of cancer. Sunitinib was evaluated for its inhibitory activity against a variety of kinases (>80 kinases) and was identified as an inhibitor of platelet-derived growth factor receptors (PDGFR α and PDGFR β), vascular endothelial growth factor receptors (VEGFR1, -2, and -3), stem cell factor receptor (KIT), Fms-related tyrosine-3 receptor (FLT3), CSF-1R, and the glial cell line-derived neurotrophic factor receptor [48]. Sunitinib inhibition of the activity of these RTKs has been demonstrated in biochemical and cellular assays, and inhibition of function has been demonstrated in cell proliferation assays. The primary metabolite exhibits similar potency compared to sunitinib in biochemical and cellular assays.

Sunitinib inhibited the phosphorylation of multiple RTKs (PDGFR β , VEGFR2, KIT) in tumor xenografts expressing RTK targets *in vivo* and demonstrated inhibition of tumor growth or tumor regression and/or inhibited metastases in some experimental models of cancer. Sunitinib demonstrated the ability to inhibit growth of tumor cells expressing dysregulated target RTKs (PDGFR, RET, or KIT) *in vitro* and to inhibit PDGFR β - and VEGFR2-dependent tumor angiogenesis *in vivo* [49].

5.1. Pharmacokinetics

Pharmacokinetic investigations in both healthy volunteers and cancer patients have shown that following a single oral dose, peak plasma sunitinib concentrations occur between 6 and 12h post dose [49]. In addition, sunitinib and SU12662 have previously been shown to display

linear pharmacokinetics and have prolonged half-lives of approximately 40 and 80h, respectively [50,51]. Sunitinib is well absorbed [51], its bioavailability is not affected by food intake [49,50,52], and no significant changes in pharmacokinetics are observed with repeat versus single dosing [49].

No differences in pharmacokinetics have been observed between healthy volunteers and cancer patients in individual studies [49,53]. However, when analyzed across multiple studies, factors such as patient status, age, gender, race, body weight, and clinical performance status may affect the pharmacokinetics of sunitinib in individuals, resulting in increased or decreased exposure to sunitinib, SU12662, or total drug.

5.1.1. Absorption

The maximum plasma concentration of sunitinib is achieved within 6–12 h, and the absolute bioavailability is unknown. The drug may be taken with or without food, since food only has a marginal effect on the exposure [52]. The inter-patient variability is large [5]. A recent case report describes a significant decrease in sunitinib exposure area-under-the-curve (AUC) in an obese patient, which might indicate that body mass index has a pronounced effect on drug exposure and might thereby explain partly the large inter-patient variability [54].

5.1.2. Distribution

The apparent volume of distribution (V_d/F) for sunitinib was 2230L. In the dosing range of 25–100mg, the area under the plasma concentration–time curve and C_{max} increases proportionately with dose. The terminal half-lives of sunitinib and its primary active metabolite are approximately 40–60 and 80–110h, respectively [5]. Binding of sunitinib and its primary active metabolite to human plasma protein *in vitro* was 95% and 90%, respectively, with no concentration dependence in the range of 100–4000 ng/mL. With repeated daily administration, sunitinib accumulates 3- to 4-fold, while the primary metabolite accumulates 7- to 10-fold. Steady-state concentrations of sunitinib and its primary active metabolite are achieved within 10–14 days.

5.1.3. Metabolism and excretion

Sunitinib is primarily metabolized by CYP3A4 to produce its primary active metabolite, SU12662, which is further metabolized by CYP3A4 into inactive metabolites [52]. The primary active metabolite comprises 23–37% of the total exposure. Data on additional enzymes involved in the metabolism are still unknown.

Sunitinib is primarily eliminated with the feces (61%), with renal elimination accounting for only 16% of the administered dose. However, there are no studies on the pharmacokinetics in patients with serious

hepatic or renal insufficiency [42]. Additionally in a case report describing two hemodialyzed patients on sunitinib therapy, the plasma concentration of the drug and its major metabolite at steady-state were comparable to patients with normal renal function [55].

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