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High-performance liquid chromatographic method for the determination of sorafenib in human serum and peritoneal fluid

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**Running title:** Determination of Sorafenib concentrations in serum peritoneal fluid

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Abstract

Purpose: Sorafenib is recommended for therapy of advanced hepatocellular carcinoma and renal cell carcinoma. Preclinical data indicate a relation between dose and antitumor efficacy. In clinical trials adverse events improve after dose reduction suggesting a dose-dependent toxicity. Given dose has a direct impact on the drug serum concentration, but the latter also can be influenced by multiple factors including interaction and metabolization. To enable the investigation of concentration related effects, an easy and sensitive assay for sorafenib drug monitoring is essential.

Methods: A high performance liquid chromatography (HPLC) analysis involving an extraction with diethyl ether followed by separation on a Pinnacle™ DB C18 column and quantitation by UV absorbance at 260 nm was established. Sorafenib concentrations in samples of serum and peritoneal fluid have been determined.

Results: The assay was validated for serum samples and is linear over the concentration range of 100 to 5000 ng/mL with a determination coefficient of >0.999. The limit of detection is 0.25 ng/mL. The intra- and inter-day coefficients of variation were below 3.03%. Sorafenib recovery in spiked probes of peritoneal fluid was above 85%. Sorafenib concentrations in 44 serum samples and 14 probes of peritoneal fluid have been determined with a mean of 3328 and 1380 ng/mL respectively (standard deviation 2267 and 659 ng/ml).

Conclusions: A sensitive and selective HPLC method for the determination of sorafenib in human serum was developed and also verified for peritoneal fluid. This method provides a useful tool for pharmacokinetic investigations as well as for therapeutic drug monitoring of sorafenib.

Keywords: Sorafenib; Hepatocellular carcinoma; HPLC; Validation; Serum concentrations; peritoneal fluid
INTRODUCTION

Sorafenib, a potent competitive oral multikinase inhibitor is suppressing the activity of several cell surface receptor tyrosine kinases, like vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor and also intracellular members of the mitogen-activated protein kinase (MAPK) signal transduction pathway [1]. Including downregulation of antiapoptotic protein this pluripotent inhibition has an effect on tumour cell proliferation, survival and tumour angiogenesis. These mechanisms have successfully been applied in the treatment of cancer in several preclinical and clinical trials [2, 3]. Today sorafenib is recommended for treatment of renal cell carcinoma [4] and currently the antitumor activity is tested for therapy of several other malignoma.

Even more convincing sorafenib has shown to prolong survival of patients with hepatocellular carcinoma (HCC) [5] and is recommended by the US National Comprehensive Cancer Network guideline for advanced HCC in Child-Pugh A or B patients [6]. If unresectable this disease is characterized by a median overall survival below one year and extrahepatic metastases, including peritoneal dissemination [7, 8]. The latter causes ascites and further complications, which can severely hamper patient’s quality of life.

Several lines of evidence suggest a dose dependent efficacy and tolerability of sorafenib. So, in vitro induction of apoptosis and inhibition of cell proliferation with sorafenib in HCC cells lines have shown to be dose dependent. A dose-related antitumour activity in a murine xenograft model of PLC/PRF/5 human HCC could be demonstrated [9]. In addition, in 26% of sorafenib recipients in a phase 3 trial dose has been reduced in order to terminate or improve adverse events [5]. Finally, pooled data suggest a relation between dose and toxicity as well as dose and efficacy [10].
Sorafenib is rapidly absorbed after oral administration and undergoes enterohepatic circulation; its elimination half-life is about 25 - 48 hours. Metabolization of sorafenib mainly occurs in the liver via oxidation by CYP3A4 (N-oxide) and via glucuronidation by UGT1A9. Pharmacokinetic interactions of sorafenib with substrates of CYP2B6, CYP2C8, CYP 2C9, UGT1A1, UGT1A9 and p-Gp, e. g. irinotecan or doxorubicin, can not be excluded (2-4). The incidence of HCC is associated with liver cirrhosis and impaired liver function, which can significantly alter the described way of drug elimination.

In order to manage possible drug interactions, to ensure an effective exposure to sorafenib and to balance interindividual pharmacokinetic variability, therapeutic drug monitoring might be a useful tool. Knowledge of sorafenib concentration in peritoneal fluid might be helpful to address further questions, as to prove the potency of clinical response in established peritoneal carcinomatosis or to evaluate a possible protection of intra-abdominal dissemination.

Here we describe a valid, rapid and sensitive HPLC-based method for the determination of sorafenib in human serum and peritoneal fluid.

MATERIALS AND METHODS

Chemicals and reagents

Sorafenib-Tosylate was kindly provided by Bayer Health Care AG (Leverkusen, Germany). The internal standard (IS) itraconazole as well as bovine serum albumin (BSA) powder (Albumin, bovine serum, fraction V, minimum 96%) were obtained from Sigma-Aldrich (Steinheim, Germany). Acetonitrile, diethyl ether, ethanol, potassium dihydrogen phosphate, anhydrous sodium carbonate, sodium hydrogen carbonate and demineralised distilled water were purchased from Merck (Darmstadt, Germany). All chemicals and solvents were of analytical or HPLC grade.
**Instrumentation**

Chromatographic analyses were carried out on a Beckman–Coulter Gold HPLC system (Krefeld, Germany) equipped with a 126 solvent delivery module, a 168 UV-VIS photodiode array detector, and a 508 autosampler. Data acquisition and analysis were performed with the Beckman System 32 Karat software. A Pinnacle™ DB C18 column, 150 × 2.1 mm, with 5 μm particle size (Restek, GmbH, Bad Homburg, Germany) protected by a C18 guard column (4 × 2 mm; Phenomenex, Aschaffenburg, Germany) was used. The mobile phase consisted of potassium dihydrogen phosphate (20 mM) and acetonitrile (35:65, v/v) (pH 6.3). The flow rate was set at 0.2 mL/min. Sorafenib and IS were determined at 260 nm. A peak controlled spectrum recording was selected with a range of 190 – 300 nm.

**Preparation of stock solutions, working solutions and quality control samples**

Stock solution of sorafenib (2.5 mg/100 mL) was prepared in ethanol and diluted for the preparation of working solutions at concentrations of 50, 100, 250, 500, 1000, 2500, 5000 and 10 000 ng/mL. The IS (itraconazole) working solution was prepared in methanol at a concentration of 5.0 mg/100 mL. Each solution was stored at -20 °C and was stable for at least three months.

For the preparation of serum standard samples, BSA solutions (5%, w/v) were spiked with sorafenib and IS (10 000 ng/mL) to obtain the above mentioned calibration and QC concentrations.

**Sample preparation**

A 250 μl aliquot of BSA standard, serum samples or peritoneal fluid was mixed with 500 μl of sodium carbonate buffer (0.1 M, pH 9.4) in a 10 mL glass tube. The IS (50 μl) was added and the solutions were briefly vortexed. Samples were extracted twice with 3 mL of diethyl ether for 5 min, followed by centrifugation at 4200 × g (5 min). The organic layers were
transferred into a glass tube and evaporated to dryness at 37 °C under a gentle stream of nitrogen. The dried residue was dissolved with 200 µl of 20 mM potassium dihydrogen phosphate in acetonitrile (1:1, v/v) and a 25 µl volume was injected into the HPLC system.

**Validation**

**Linearity**

Complete calibration curves were analysed in duplicate on three separate days. A linear regression with a weighting factor of $1/(\text{peak height ratio})^2$ was used to plot the peak height ratio (height of sorafenib/height of IS) versus the corresponding sorafenib concentration. Slopes, y-intercepts, and correlation coefficients ($r^2$) were calculated.

**Accuracy and precision**

Intra-day accuracy and precision of the method were determined by measuring 12 replicates of each QC concentration on the same day. This procedure was repeated on 12 different days to test the inter-day accuracy and precision. Accuracy was calculated as the relative error of the nominal concentration. Precision was expressed as the coefficient of variation and obtained by analysis of variance (ANOVA) for each test concentration using the analytical run as the grouping variable.

**Sensitivity**

The limit of detection (LOD) was defined as the lowest detectable concentration level resulting in a signal-to-noise ratio of three [11]. For the lower limit of quantitation (LLQ), accuracy (deviation from nominal concentration) and precision (relative standard deviation) should not exceed 20% [12]. The upper limit of quantitation (ULQ) was arbitrarily set at 20 000 ng/mL.
**Recovery**

The recovery of sorafenib and IS in serum were performed in quadruplicate by comparing the analytical results for extracted QC samples with unextracted standards presenting 100% recovery. Samples of peritoneal fluid from five patients without sorafenib medication were spiked with increasing concentrations of 100, 250, 500, 1000 and 5000 ng/ml sorafenib. Recovery in spiked ascites was determined in parallel with the calibration of serum samples.

**Specificity and selectivity**

The specificity of the method was indicated by the absence of any endogenous interfering peaks. For testing method selectivity, drug-free BSA solutions were spiked with therapeutic concentrations of diazepam, fentanyl, furosemide, metamizole sodium, pantoprazole, propanolol, spironolactone, tilidine, tramadole, and torasemide.

**Stability**

Stability studies of sorafenib in spiked BSA solutions were performed with QC samples. Four series of each QC concentration were stored for 30 days at -20 °C, 7 days at -20 °C including 3 freeze-thaw cycles, 7 days at 4 °C, 24 h at room temperature and 60 min at 56 °C. Long-term stability was determined following three months of storage at -20 °C. After each storage period the concentration of sorafenib was analysed and compared to freshly prepared QC samples.

**Analysis of patient samples**

Blood samples from patients treated with sorafenib for the therapy of HCC were taken after ingestion of 400 mg sorafenib twice every day (BID). Serum samples were obtained by a
standardised procedure. Samples of peritoneal fluid were collected in case of paracenteses. Simultaneously cell count, lactate dehydrogenase, cholesterol, triglycerid, glucose- and protein concentrations were determined. All samples were separated by centrifugation at 4200 \( \times g \) for 10 min at 20 °C and immediately stored at -20 °C until further analysis. Determination of sorafenib concentrations in patients’ peritoneal fluid were performed in the same assay and with the calibration of serum samples.

**Calculation and data analysis**

All statistical calculations were performed with Statistical Product and Service Solutions (SPSS) for Windows, version 17.0 (SPSS, Chicago, Illinois, USA). T-tests of mean concentrations and correlations were considered statistically significant at \( p \)-values \( \leq 0.05 \).

**RESULTS**

**Sample preparation, chromatography and detection**

Representative HPLC-chromatograms of a drug-free BSA sample (blank) and a BSA sample spiked with 2500 ng/mL sorafenib and the IS are shown in Figure 1. Retention times for sorafenib and IS were 4.65 and 6.48 min. No interfering endogenous peaks were detectable in the blank sample. In this assay the chromatographic run-time was 10 min.

**Linearity and recovery**

The linearity of the method was evaluated over a concentration range of 100 – 5000 ng/mL. Using the ratios of observed peak heights for sorafenib and IS in six spiked BSA samples analysed in duplicate, the calibration curves showed a correlation coefficient (\( r^2 \)) of >0.999 with a mean slope of 3806.70 ± 109.62 and a mean \( y \)-intercept of 23.260 ± 3.662. Highest
recovery values with 97.66 ± 1.67% for sorafenib and 88.29 ± 2.04% for IS were achieved at pH 9.4.

**Accuracy and precision**

Intra- and inter-day accuracy and precision data of QC samples are shown in Table 1. Intra-day accuracy, expressed as the relative error (RE; in percent) ranged from 0.22 to 0.77%. Intra-day precision, expressed as the coefficient of variation (CV; in percent) was 0.60 to 3.03%. The inter-day accuracy and precision were less than -3.55 and 2.62%, respectively.

**Sensitivity and specificity**

The LOD of sorafenib in plasma was determined at 0.25 ng/mL, and LLQ was established at a concentration of 50 ng/mL. ULQ was arbitrarily set at 20 000 ng/mL.

Serum samples from both patients treated with sorafenib and healthy individuals were devoid of interference near the retention times of sorafenib and IS under the used chromatographic conditions.

The analysis of spiked drug-free BSA samples diazepam, fentanyl, furosemide, metamizole sodium, pantoprazole, propanolol, spironolactone, tildine, tramadole, or torasemide showed no interference neither with the extraction procedure nor with the analytical method.

**Stability**

Freeze-thaw stability of QC in spiked BSA samples was demonstrated after 7 days of storage at -20 °C including three freeze-thaw cycles. Sorafenib was also stable after 30 days at -20 °C, 7 days at 4 °C, 24 h at room temperature, 60 min at 56 °C and after 3 month at -20 °C (Table 2). RE and CV were less than 2.75% and 3.73%, respectively.
**Sorafenib in peritoneal fluid**

Clinical parameters of spiked peritoneal fluid are shown in table 3, representing a broad range of clinical conditions, as demonstrated by leukocyte count, glucose concentration and osmolarity. Peak heights in chromatography were not significantly different for samples of blank serum or peritoneal fluid, when spiked with either 100, 250, 500, 1000 or 5000 ng/ml sorafenib (< 10%, data not shown). For recovery of sorafenib in spiked samples of ascites a maximum deviation of 14% in concentration has been determined and this has been most pronounced in samples with the highest concentrations. Mean concentrations have been within 10 % of the expected value (Figure 2).

**Analysis of patient samples**

Fourtyfour serum samples were taken after ingestion of 400 mg sorafenib BID. Determined sorafenib concentrations ranged from undetectable to 8833 ng/mL, with a mean of 3747 ng/mL and a of median 3881 ng/mL. The standard deviation was calculated as 2267 ng/ml. Only two concentrations were below 100 ng/ml and altogether 6 below 1000 ng/ml. Most concentrations (28/44) were in the range of 1000 to 6000 ng/ml, three, two and two concentrations were between 6000 to 7000, 7000 to 8000 and 8000 to 9000 ng/ml, respectively.

In fourteen samples of peritoneal fluid sorafenib concentrations had a much smaller range of 510 to 2757 ng/ml, with a mean of 1380 ng/ml, a median of 1235 ng/mL and a standard deviation of 659 ng/ml. Half of the samples had a concentration between 1100 and 1900 ng/mL. Ascites and serum sorafenib mean concentrations were significantly different (p <0.01) and are illustrated in figure 3.

**DISCUSSION**
Here we established an easy and reproducible method for determination of sorafenib concentration in serum and peritoneal fluid. This assay facilitates a precious determination of sorafenib at least in the range of 50 to 20 000 ng/ml. No interference with other compounds was detected. Sorafenib concentrations were quantified in forty-four serum samples from patients treated with this tyrosine kinase inhibitor. In addition, relevant amounts of sorafenib were detected in fourteen probes of peritoneal fluid. The median of the later was below the concentrations in serum, but still in the range previously described in human plasma.

Only a few methods for quantification of sorafenib in human or mouse plasma by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [13-15] were reported so far. Although HPLC-MS/MS techniques require only small sample volumes and minimal sample preparation they are expensive and not generally available. Up to now, only two HPLC-UV assays were published for the determination of sorafenib either in mouse serum [16] or in human plasma [17] using sample preparation by protein precipitation with acetonitrile in combination with liquid-liquid extraction. Avoiding this complex preparation, in the here described assay, a 250 µL aliquot of human serum following liquid-liquid extraction with diethyl ether was used. Flow rate and the chromatographic run-time were reduced to 0.2 mL/min and 10 min, respectively, making this assay faster and more cost-effective. Most important for our HPLC method we were able to demonstrate an improved sensitivity.

In addition, we tested the assay for determination of sorafenib concentration in peritoneal fluid. Here, recovery and precision of expected and determined concentration were satisfactory for clinical use over a spectrum of sorafenib concentrations, clearly broader than the concentrations recovered in patient plasma samples. As reflected by standard laboratory parameters including leukocyte count, used probes of peritoneal fluid represent a broad range of clinical conditions, including a spontaneous bacterial peritonitis. Therefore, the assay,
which was introduced here, makes it easy to determine sorafenib concentration in blood and ascites. Furthermore both can be tested simultaneously.

Some data suggest a correlation between prescribed drug doses and clinical efficacy or adverse events [10]. In a dose escalation trial, drug related grade 3 adverse events were limited to the higher doses (400 and 800 mg BID) but did not occur at doses of 100 or 200 mg BID [18]. Also a dose-dependent relationship for certain adverse events was observed and due to an unacceptable incidence rate of dose limiting toxicity at 600 mg BID in a phase I study 400 mg BID was chosen for maximum tolerated dose [19].

Although dose triggers drug concentration, the latter is also influenced by several other factors, like drug interaction, impaired liver function, inhibited or induced metabolization and compliance. Especially in HCC patients an impaired liver function is common, but only very limited data on pharmacokinetics for Child-Pugh A or B patients exist from a phase I trial in 14 Japanese patients and 22 patients in a phase II trial [20, 21], but none for Child-Pugh C and effect on liver function including cytochrome p450 activity can be extremely heterogeneous in patients with the same stage of liver cirrhosis.

Up to now, only few data are published on sorafenib concentrations and mainly mean or median results of AUC and maximum concentrations are mentioned. This makes it difficult to investigate a correlation of effects, positive or negative, to drug concentration. So, in a phase I trial 9 subjects receiving 400 mg sorafenib BID had a mean $C_{max}$ at day seven of 9.90 mg/L with a standard deviation of 1.52 and a range of 5.63 – 23.0 mg/L [19]. Maximum concentrations are more difficult to obtain in clinical practise compared to trough levels. Here we could show a broad range of individual sorafenib trough concentrations in serum samples. Intra- and interindividual differences as well as their impact on antitumor activity and drug safety have to be investigated in prospective trials. We also could demonstrate significant different sorafenib concentrations in peritoneal fluid. Drug concentration in this body
compound might be influenced by additional determinants. These include but are not limited to cause of ascites (exsudative and transudative), volume and time of development. Nevertheless, observed sorafenib concentrations are within a restricted spectrum. A potential protective or therapeutic effect for peritoneal carcinosis still has to be evaluated.

The described assay might help to evaluate the impact of potential drug interactions, especially as studies for the use of sorafenib in combination with e.g. irinotecan, evorolimus or bortezomib for treatment of HCC have already started [22-24].

**CONCLUSIONS**

We have developed and validated a simple, selective and reliable analytical assay for the determination of sorafenib in human serum which can readily be used in a standard laboratory. The practicability of the assay is demonstrated by serum levels of 44 patient samples. In addition, we were able to show that the method is also qualified to quantify sorafenib concentrations in peritoneal fluid, and results of 14 samples are presented. To our knowledge for the first time, significant sorafenib concentrations within the range observed in plasma have been demonstrated in peritoneal fluid. So far, its clinical implications remain to be determined. In particular, serum showed a broad range of sorafenib concentrations. Further and prospective investigations are necessary and warranted for evaluation of concentration dependent effects.

The described assay can already be useful as a tool for therapeutic drug monitoring in order to optimise drug dosage on an individual basis. It can also be used for pharmacokinetic and pharmacodynamic studies of sorafenib.
Tables

Table 1: Intra- and inter-day accuracy (RE; %) and precision (CV; %) for the determination of sorafenib in spiked serum samples by HPLC; a observed concentrations as a mean of 12 ± SD

<table>
<thead>
<tr>
<th>Nominal concentration (ng/mL)</th>
<th>Observed concentration a (ng/mL)</th>
<th>RE (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-day (n = 12)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>99.49 ± 3.01</td>
<td>0.51</td>
<td>3.03</td>
</tr>
<tr>
<td>2500</td>
<td>2494.5 ± 37.57</td>
<td>0.22</td>
<td>0.60</td>
</tr>
<tr>
<td>5000</td>
<td>4961.3 ± 121.6</td>
<td>0.77</td>
<td>2.45</td>
</tr>
<tr>
<td><strong>Inter-day (n = 12)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>103.5 ± 2.46</td>
<td>-3.54</td>
<td>2.38</td>
</tr>
<tr>
<td>2500</td>
<td>2564.1 ± 66.89</td>
<td>-2.57</td>
<td>2.61</td>
</tr>
<tr>
<td>5000</td>
<td>5108.0 ± 129.1</td>
<td>-2.16</td>
<td>2.53</td>
</tr>
</tbody>
</table>
Table 2: Stability of three sorafenib concentrations in spiked 5% BSA solution, at different temperatures, storing conditions, and durations as listed in column 1; \(^{a}\) Mean of observed concentrations; \(n = 4;\)

<table>
<thead>
<tr>
<th>Nominal concentration (ng/mL)</th>
<th>100</th>
<th>2500</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days at -20 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (ng/mL)(^{a})</td>
<td>104.7</td>
<td>2602.5</td>
<td>5187.3</td>
</tr>
<tr>
<td>SD</td>
<td>1.42</td>
<td>28.10</td>
<td>25.57</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-4.65</td>
<td>-4.09</td>
<td>-3.75</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.36</td>
<td>1.08</td>
<td>0.49</td>
</tr>
<tr>
<td>7 days at -20 °C with 3 freeze-thaw cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (ng/mL)(^{a})</td>
<td>102.5</td>
<td>2620.3</td>
<td>5030.0</td>
</tr>
<tr>
<td>SD</td>
<td>3.34</td>
<td>85.17</td>
<td>41.75</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-2.15</td>
<td>-4.83</td>
<td>-0.60</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.76</td>
<td>3.25</td>
<td>0.83</td>
</tr>
<tr>
<td>7 days at 4 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (ng/mL)(^{a})</td>
<td>101.6</td>
<td>2568.0</td>
<td>5025.5</td>
</tr>
<tr>
<td>SD</td>
<td>3.34</td>
<td>26.71</td>
<td>186.9</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-1.60</td>
<td>-2.72</td>
<td>-0.51</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.29</td>
<td>1.04</td>
<td>3.72</td>
</tr>
<tr>
<td>24 h at room temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (ng/mL)(^{a})</td>
<td>103.0</td>
<td>2535.8</td>
<td>4966.5</td>
</tr>
<tr>
<td>SD</td>
<td>3.75</td>
<td>63.12</td>
<td>149.5</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-2.95</td>
<td>-1.39</td>
<td>0.67</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.64</td>
<td>2.49</td>
<td>3.01</td>
</tr>
<tr>
<td>60 min at 56 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (ng/mL)(^{a})</td>
<td>107.3</td>
<td>2431.3</td>
<td>5080.0</td>
</tr>
<tr>
<td>SD</td>
<td>2.96</td>
<td>5.59</td>
<td>120.4</td>
</tr>
<tr>
<td>RE (%)</td>
<td>-7.30</td>
<td>2.75</td>
<td>-1.60</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.76</td>
<td>0.23</td>
<td>2.37</td>
</tr>
</tbody>
</table>
Table 3: Standard laboratory parameters of 6 samples of peritoneal fluid spiked with sorafenib:

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>sample number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td>235</td>
</tr>
<tr>
<td>Lactat dehydrogenase</td>
<td>U/L</td>
<td>37</td>
</tr>
<tr>
<td>Total protein</td>
<td>g/dL</td>
<td>0.8</td>
</tr>
<tr>
<td>Albumine</td>
<td>g/dL</td>
<td>0.3</td>
</tr>
<tr>
<td>Cholesterin</td>
<td>mg/dL</td>
<td>8</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg/dL</td>
<td>114</td>
</tr>
<tr>
<td>Weight density</td>
<td>g/ml</td>
<td>1.011</td>
</tr>
<tr>
<td>Cell count/leukocytes</td>
<td>n x 1000/µL</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Figures

Figure 1: Representative HPLC-chromatograms (260 nm) of (A) drug-free 5% BSA sample (blank) and of (B) 5% BSA sample spiked with 2500 ng/mL sorafenib (SFB) and the internal standard itraconazole (IS). Retention-times are presented in minutes and amount of absorbance in milli-absorbance-units (mAU).

Figure 2: Calculated and determined sorafenib concentrations detected in spiked peritoneal fluid.

Figure 3: Sorafenib concentrations (in ng/mL) in serum and ascites determined by HPLC.
Literature


