Analytical aspects of therapeutic drug monitoring: the case of sunitinib and its geometric isomerism

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Background
Sunitinib malate (SUT) is an oral multi-target tyrosine kinase inhibitor, recently approved by FDA for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumour. Actually, small molecules like SUT are used at fixed doses ignoring the possible need for dose individualization. Due to the high inter-variability on the pharmacokinetics of these therapies, this could result in a suboptimal treatment or an excessive toxicity. In this scenario, therapeutic drug monitoring (TDM) could be a good approach in order to obtain a tangible benefit in terms of improvement of therapeutic effect. In fact, SUT fulfilled many of the specific criteria required for the application of TDM such as a high inter-individual pharmacokinetic variability and evidences of a relationship between concentration and pharmacological effects. Nonetheless, in order to apply the TDM approach in the clinical practice it is necessary also to have a rapid, sensitive, specific, and reproducible analytical method. However, this goal is difficult to achieve due to a peculiarity of SUT represented by its isomerisation in presence of light: the Z-isomer is the active compound and converts to the E-isomer, which is the inactive one. This phenomenon does not cause as much pharmacological but rather analytical issues and most of the methods already published for the quantification of SUT require light protection conditions. Unfortunately, these conditions are unsuitable for clinical application and, therefore, we planned to overcome this problem by inducing the reversion of the E- to Z-isomer exploiting high temperatures. The aim of this work was to create a more standardised experimental conditions for an HPLC-MS/MS method for the quantification of SUT and its main metabolite, SU12662, suitable for clinical practice and for TDM.
Methods
The study of the Z-E isomerisation kinetics of SUT and SU12662 has been conducted in plasma, after protein precipitation, by illuminating the samples with a LED lamp for increasing periods of time. A HPLC-MS/MS method, specifically developed and validated for this study, has been applied for the analysis of these samples: the analytes were separated on a Synergy Fusion RP C18 (4 μM, 80 Å, 2 x 50 mm) and the HPLC system consisted of a SIL-20AC XR auto-sampler and LC-20AD UFLC XR pumps (Shimadzu, Tokyo, Japan). The detector was an API 4000 triple quadrupole mass spectrometer AB SCIEX (Massachusetts, USA) and the data were processed with the software Analyst 1.5.2.
Results
When we exposed the SUT samples to the LED light, the formation of E-isomer did not reach completion with nearly 60% formed after one hour. An analog equilibrium achievement has been noticed for SU12662 but its E-isomer reached a maximum of only 20%. Then, the E- to Z-isomer reconversion kinetics has been investigated in dark conditions leaving the samples into the autosampler set at different temperatures (4 and 25°C). The reconversion rate resulted increased by higher temperatures (after 5 h in dark condition at 25°C the Z-SUT was 99% while at 4°C it was only 55%), thus indicating a thermal reversion. Based on these data, in order to decrease the E- to Z-isomer reconversion time, we put the samples in a heated-water bath at different temperatures (from 40° to 90°C) just before the analysis, reaching the maximum percentage of the Z-isomer for both SUT and SU12662 in only 5 min with temperature equal or higher than 70°C.
Conclusions
The developed method does not require the protection from the light during the sample preparation but only a heating step with a heated water bath at 70°C for 5 min. The incubation of the samples, just before their introduction into the autosampler for the analysis, allows the rapid reconversion of the E-isomer, formed during the sample preparation, to the Z-form. This approach strongly facilitates the handling of the samples rendering this method more feasible for TDM.