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Bioanalysis in clinical development of tasquinimod using liquid chromatography/tandem mass spectrometry

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ABSTRACT

Tasquinimod (ABR-215050) is an oral drug in clinical development for treatment of patients with castrate resistant prostate cancer. This paper describes a method for the determination of tasquinimod in human plasma. The method is based on liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) using stable isotope labeled tasquinimod as internal standard (IS). The plasma samples were processed by protein precipitation using acidic acetonitrile containing the IS. The precipitated samples were centrifuged and the supernatant was injected directly into the LC–MS/MS system. Chromatographic separation was performed on a reversed phase column using fast gradient elution, with a total run cycle time of 4 min. The method was validated with respect to accuracy, precision, dynamic range, lower limit of quantification, selectivity and robustness. Furthermore, the stability of tasquinimod in spiked plasma, in processed extracts and in incurred samples was thoroughly studied. The method was validated in the range of 1.0–2400 nmol/L, defining the lower and upper limits of quantification. The repeatability, reproducibility and overall bias were 1.5–7.1%, 3.5–7.4%, and 1.3–4.7%, respectively, in the range of 1–2000 nmol/L. Excellent selectivity was demonstrated in the validation, as well as in study samples from both healthy volunteers and cancer patients. Robustness was demonstrated by the calculated carry-over as low as 0.06%, and by an incurred sample reproducibility (ISR) experiment where 97% of the reanalyzed samples fulfilled the acceptance criteria of 20% deviation from initial analysis result. Also, tasquinimod was found to be stable in all investigated matrices, including in incurred samples. In an incurred sample stability (ISS) investigation, tasquinimod was demonstrated to be stable for 24 months, and 97% of the reanalyzed samples were within 20% from the initial analysis result. In conclusion, the method was demonstrated to be accurate, precise, robust and reliable for the determination of tasquinimod. The method was successfully used in several clinical studies for the support of pharmacokinetic and pharmacodynamic evaluations.

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1. Introduction

Tasquinimod (ABR-215050, 1,2-dihydro-N,1-dimethyl-4-hydroxy-5-methoxy-2-oxo-N-(4-trifluoromethylphenyl)-3-quinolinecarboxamide, CAS number 254964-60-8) is an orally active small molecule which has shown promising effects in several preclinical models of cancer [1,2]. The drug has been shown to act as an anti-angiogenic agent [3] by mechanisms that e.g. upregulates thrombospondin-1 [4], hypothesized to be due to interactions with the pro-inflammatory protein S100A9 [5]. Tasquinimod has been selected as a candidate drug for the treatment of patients with castrate resistant prostate cancer (CRPC), and has been shown to be safe and well tolerated in phase I clinical studies [6]. Furthermore, robust efficacy and safety data from a placebo controlled pivotal phase II trial in CRPC patients [7], demonstrating significantly prolonged progression-free survival (PFS) in treated patients as compared to placebo were recently presented. In early 2011, a global multi-site phase III study of tasquinimod treatment of CRPC patients was initiated. The clinical development of tasquinimod necessitated the development of a sensitive, selective, and robust bioanalytical method for the determination of the drug in human plasma. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was the preferred technology. No previous methods for the determination of tasquinimod in human plasma have been reported. However, for

Abbreviations: CRPC, castrate resistant prostate cancer; LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; IS, internal standard; SRM, selected reaction mode; ESI, electrospray ionization; TFA, trifluoroacetic acid; QC, quality control; LLOQ, lower limit of quantification; RSD, relative standard deviation; ISR, incurred sample reproducibility; ISS, incurred sample stability; EBF, European Bioanalysis Forum.

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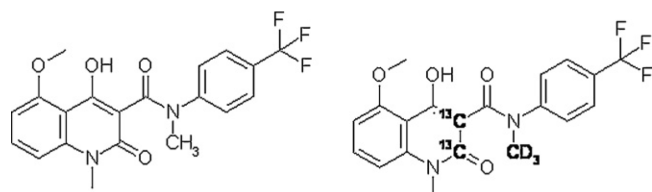

Fig. 1. Chemical structure of tasquinimod (left) and the stable isotope labeled internal standard (IS) (right).

the related quinoline-3-carboxamide analogue, laquinimod, there are two bioanalytical methods described in the literature [8,9], of which the latter is based on LC–MS/MS. Our new method for tasquinimod was mainly validated according to current regulatory guidelines for the validation of bioanalytical methods [10,11] with extended studies on the stability of tasquinimod in spiked and incurred samples. The aim of this paper is to describe the development and validation of a method for the determination of tasquinimod in human plasma.

2. Materials and methods

2.1. Chemicals and reagents

Tasquinimod and the stable isotope labeled internal standard (Fig. 1) with purities of >99.5% were synthesized at Active Biotech Research in Lund. Lab-scan Ltd. (Dublin, Ireland) was the supplier of HPLC grade methanol and acetonitrile. Ammonia (25% NH_3 (aq), pa) was purchased from AnalaR, BDH Ltd. (Poole, UK). Trifluoroacetic acid (TFA) and potassium hydroxide (KOH) was purchased from Sigma (St. Louis, MO, USA). Deionized water was produced on site with a Milli-Q water system from Millipore (Bedford, MA, USA).

2.2. Equipment

For sample preparation, 1 mL deep 96-well polypropylene plates (Porvair Science, UK) were used. The plates were sealed with a pierceable sealing aluminum foil (ABgene, Epsom, UK). The liquid chromatography system consisted of an HP1100 binary pump from Hewlett Packard (Avondale, PA, USA) and an autosampler from CTC analytics (Zwingen, Switzerland). The CTC autosampler was equipped with a 6-port injection valve and a 20 μL stainless steal loop. The samples were analyzed by fast gradient reversed phase chromatography using a Symmetry Shield RP18, 30 mm × 2.1 mm, 3.5 μm particles (Waters Corp, Milford, MA, USA). For detection, an API 3000^{TM} triple quadropole mass spectrometer from AB Sciex (Foster City, CA, USA) equipped with an electrospray interface was used. The Analyst software from AB Sciex (Foster City, CA, USA) was used to control the LC–MS/MS system, as well as for the tuning, calibration and evaluation of MS data.

2.3. Preparation of spiked plasma samples

Stock solutions of tasquinimod were prepared by dissolving accurately weighed amounts of pure tasquinimod in 0.1 M NH_3 (aq). Working solutions were prepared by further diluting the stock solutions in 0.025 M NH_3 (aq). Two sets of stock solutions were prepared to counter-act weighing error. These working solutions were added to drug-free human plasma for preparation of the calibration and QC samples. Eight calibration samples (S1–S8) with the concentrations 1.0, 2.9, 9.2, 27.1, 85.6, 254, 804 and 2370 nmol/L, respectively, were prepared. Four concentration levels of quality control (QC) samples with the concentrations 1.0, 2.5, 70 and 2000 nmol/L were prepared, referred to as QC_LLOQ, QC_L, QC_M, and QC_H, respectively. The analyte has been shown to be stable in alkaline solution at 8 °C for at least four weeks. A stock solution of the internal standard (IS) was prepared by first dissolving accurately weighed IS in 0.1 M NH_3 to a concentration of 0.1 mg/mL and then further diluting the resultant solution to a concentration of 100 nmol/L in 0.2% TFA in acetonitrile.

2.4. Analytical method

The method protocol involved protein precipitation followed by LC–MS/MS analysis. First, for each sample, a plasma volume of 100 μL was transferred to 96-deep well plates. Subsequently, a volume of 200 μL of the IS working solution was added for precipitation of plasma proteins. After sealing and mixing, the deep well plate was centrifuged in order to pellet the precipitated proteins. The supernatant was directly injected in a gradient liquid chromatography system, using a 10 μL injection volume with the injector configured in front flush injection mode with a 20 μL external loop. In order to minimize analyte carry-over, the autosampler syringe, injection port, valve and rotor were thoroughly washed after each injection with two wash solutions. The first solution was 1% KOH in MeOH:H_2O (1:1). The second was an acetonitrile solution with 0.2% TFA. The mobile phase consisted of a mixture of two solutions; A and B. A consisted of 0.01% TFA in water and B consisted of 0.01% TFA in methanol. The LC gradient was run from 95:5 to 0:100 of mobile phase A:B in 1 min, was kept constant from 1 to 2 min at 0:100 and was returned to 95:5 mobile phase A:B for 0.1 min. Finally, the column was re-equilibrated for 1.9 min prior to the next injection, giving a total run cycle time of 4 min. The LC flow rate was 0.3 mL/min. The MS electrospray ionization (ESI) interface was operated in positive mode with an ion source temperature of 350 °C, a spray potential of 4500 V and a declustering energy of 21 eV. Highly purified nitrogen (Air Liquide, Paris, France) was used as nebulizer, curtain and collision gas. Collision energy was set to 23 eV for collision-induced fragmentation of the analytes. The selected reaction mode (SRM) transitions were set to m/z 407.1 → 232.1, and 412.1 → 234.1 for tasquinimod and the IS, respectively. The dwell times were 100 and 50 ms for tasquinimod and the IS, respectively. Unit resolution was used for both quadrupoles.

2.4.1. Method development

The LC pump configuration was modified to be able to run fast gradient elution. In order to optimize the signal-to-noise ratio of the tasquinimod response, methanol versus acetonitrile and formic acid versus TFA were evaluated as mobile phase constituents. Also, the concentration of the chosen additive was evaluated. Furthermore, ESI in both positive and negative mode was considered. Optimization of MS parameters was performed during a 5 μL/min infusion of 200 nmol/L of ABR-215050 in a methanol:water (50:50) solution. Injection mode and minimization of analyte carry-over effects were evaluated for the autosampler. In the optimization of the sample preparation, ethanol, methanol and acetonitrile were evaluated as precipitation agents.

2.4.2. Method validation

2.4.2.1. The lower limit of quantification and selectivity. The lower limit of quantification (LLOQ) was defined as the lowest tested concentration that could be quantified with a precision of <20% and an accuracy of 80–120%. Also, the analyte signal at LLOQ should be at least five times the signal of blank plasma when carry-over effects are taken into consideration. Endogenous interference with the analyte and the IS SRM transitions were investigated by analyzing drug free plasma from six healthy volunteers. The samples were prepared according to the method protocol except that no IS was added.

2.4.2.2. Accuracy, precision and dynamic range. The accuracy and the intra-day as well as the inter-day precision of the method were determined by analyzing four concentration levels of tasquinimod (QC_LLOQ, QC_L, QC_M and QC_H) in five replicates at each level and at three different occasions along with a calibration curve containing the eight calibration samples S1–S8. The accuracy was calculated for each concentration level as the measured concentration in relation to the nominal concentration. The intra-day precision was calculated as the relative pooled standard deviation. The inter-day precision was calculated as the relative standard deviation (RSD) for all replicates at each concentration level. The optimal curve fit of the calibration curves was evaluated by regression analysis. The signal for the LLOQ calibration sample was used to establish integration parameters in each analytical batch. Peak area and peak height were evaluated with linear and quadratic curve fittings using various weightings.

2.4.2.3. Robustness. Two batches of analytical columns were evaluated with respect to retention times and peak shape. The peak shape robustness was evaluated by comparing injection volumes of 8, 10 and 12 μL. The resulting chromatograms were visually inspected. Carry-over was estimated by running high concentration samples (QC_H) followed by solvent blanks. The carry-over was calculated by dividing the signal of the first solvent blank with the signal of high concentration sample. In order to investigate the dilution integrity, spiked samples were diluted up to 10 times with drug-free human plasma and the results were compared with nominal concentrations.

2.4.2.4. Stability of spiked samples. The short-term stability of tasquinimod in spiked plasma was investigated at 5 and 1900 nmol/L. The bench top stability at ambient temperature was studied for 6 h. The freeze/thaw stability in −20 °C was studied during three cycles. The autosampler storage stability of processed plasma samples at ambient temperature was studied for up to 24 h. The long-term stability of tasquinimod in spiked plasma at −20 °C was investigated for 30 months, using a non-conventional approach. The design involved sequential preparation and storage of four sets of calibration samples (S1–S8) several times during a 30 month period. At the end of this period all of these samples were analyzed on a single occasion, using only the freshly prepared calibration samples as calibrators for quantification. In this way the stability of the S1–S8 samples was monitored after 0, 5, 12 and 30 months of storage. The analyte concentrations were approximately those given for S1–S8 in Section 2.3.

2.4.2.5. Incurred sample re-analysis. Incurred sample reproducibility (ISR) experiments was performed in a clinical study of CRPC patients given repeated doses of 0.25, 0.5 or 1 mg of tasquinimod. The ISR was performed two days after the initial analysis, and the samples were stored at −20 °C. The ISR was performed for 29 samples in the concentration range of 1.6–1050 nmol/L (average 373 nmol/L), thus covering the entire calibration range. Furthermore, a single point long-term incurred sample stability (ISS) investigation of 38 incurred samples from a study of healthy volunteers was performed after two years of storage at −70 °C. The healthy subjects had been administered either a single dose of 3 mg or repeated doses of 1 or 2 mg tasquinimod. The selection of incurred samples for re-analysis was performed after initial analysis and was aimed at including a broad range of analyte concentrations as well as patients, including samples that were close to the maximum plasma concentration and samples from the late elimination phase. The recommended acceptance criteria by European Bioanalysis Forum (EBF) for ISR were applied, i.e. that 2/3 of the repeat values should be within 80–120% of the original value [12].

2.5. Clinical application

The developed and validated method was applied in several clinical studies of tasquinimod in healthy volunteers and in CRPC patients. Herein, we present the results from a dose escalation study in healthy volunteers administered with a single oral dose of 0.5, 1.5 and 3 mg, respectively. Each dose group consisted of four subjects and plasma samples were collected from each subject at 0, 0.17, 0.33, 0.75, 1.5, 3, 9, 24, 48 and 96 h, after the administration of tasquinimod. The study was approved by the local ethical committee and written informed consent was obtained from each subject.

3. Results

3.1. Method development

In order to reduce the dwell volume of the LC system, a binary Agilent 1100 pump was chosen in favor of a quaternary Agilent 1100 pump. For the same reason, the pump mixer was also removed. Acidic methanol was superior to acidic acetonitrile as a mobile phase with respect to signal to noise ratio. TFA was preferred over formic acid as a mobile phase additive as it enhanced the signal response for tasquinimod, and the response was further increased when the concentration of TFA was decreased from 0.1% to 0.01%.

In positive full scan mode, the spectrum of ABR-215050 revealed a dominating protonated molecule $[M+H]^+$ of m/z 407.1 amu which was chosen as precursor ion. However, several cation and solvent adducts were also visible. In MS/MS mode, the formation of product ions revealed a major fragment at m/z 232.1, which was interpreted as the quinoline-3-carboxy moiety (Fig. 2). In SRM optimization of various transitions, this fragment was found to gain superior sensitivity for the determination of tasquinimod. Thus, the SRM transition chosen was m/z 407.1 → 232.1. For the IS, the corresponding transitions m/z 412.2 → 234.1 was chosen using the same parameters as for unlabeled tasquinimod.

When using a 20 μL injection loop partially filled with an injection volume of 10 μL using the autosampler in conventional back flush injection mode, the chromatographic peaks were distorted. Therefore, the injector was configured in front flush mode, which resulted in an improved peak shape. A two-solvent wash consisting of a strongly alkaline methanol–water solution followed by an acidic organic solvent were necessary to achieve a sufficiently low carry-over of analytes and sample background.

An effective protein precipitation and a minimum of organic solvent addition was obtained by using two parts of 0.2% TFA in acetonitrile to one part of plasma. The addition of TFA in the acetonitrile was found to enhance protein precipitation.

3.2. Method validation

3.2.1. LLOQ and selectivity

The LLOQ was determined to be 1 nmol/L. Typical chromatograms for blank plasma vs. plasma spiked with tasquinimod at the LLOQ are compared in Fig. 3. As is shown, negligible endogenous interfering peaks are observed and hence, the selectivity was considered satisfactory. The selectivity for the IS SRM transition was similar and satisfactory.

3.2.2. Accuracy, precision and dynamic range

Precision and accuracy were within acceptance criteria of ±15% for all analyzed concentrations, as is shown in Table 1. The dynamic

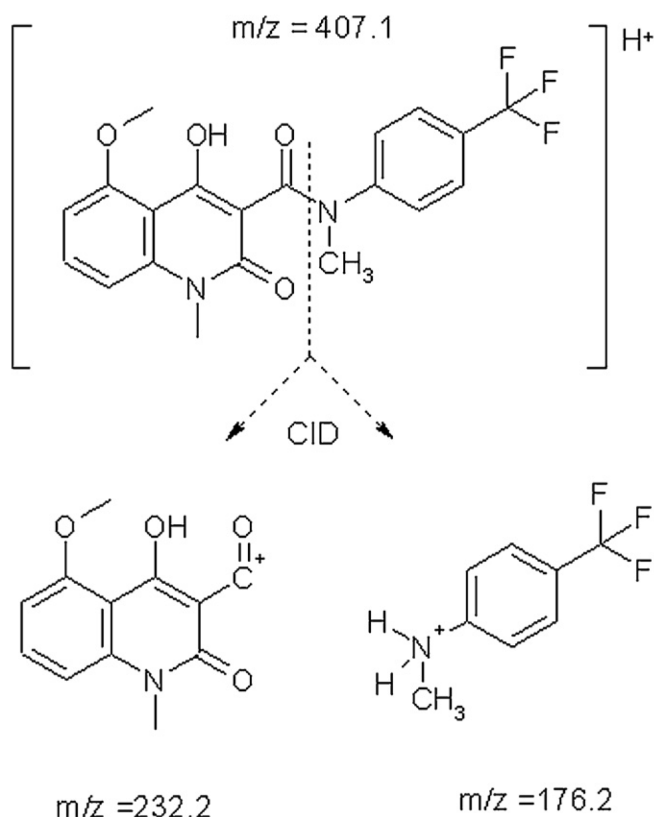

Fig. 2. Proposed collision induced decomposition (CID) fragmentation of tasquinimod.

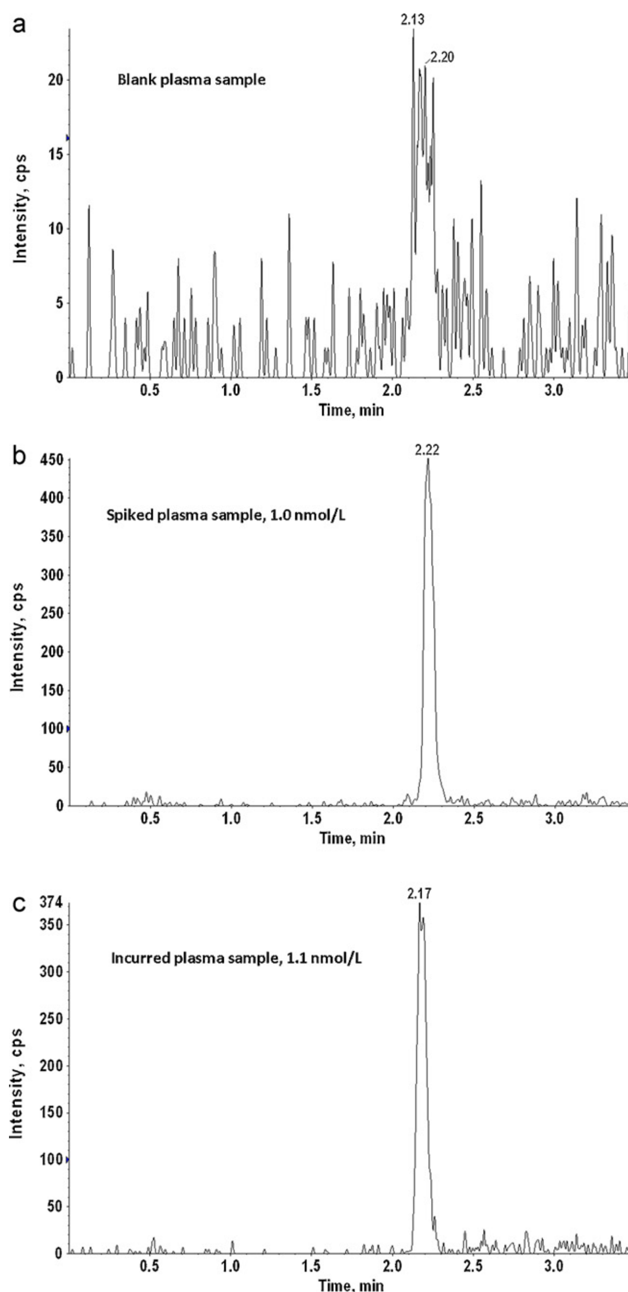

Fig. 3. Selectivity of the SRM transition *m/z* 407.1 to 232.1 for tasquinimod. Blank plasma (upper) as compared to plasma spiked with tasquinimod at the LLOQ (1 nmol/L, middle) and incurred plasma obtained from a cancer patient (1.1 nmol/L, lower).

range was set to 1–2400 nmol/L. The best fit of the calibration curve was obtained by using a quadratic curve fit with the weight $1/C^2$ (C, analyte concentration) using peak area ratio. In all three validation batches, the back-calculated values were <5% for all calibration samples and the obtained correlation coefficients (R^2) were over 0.999.

3.2.3. Robustness

No significant column to column variations were observed. The carry over was calculated to 0.06% for the highest calibration sample. No alteration of the peak shape was seen when the injection volume was increased from 8 to 12 μL, indicating that 10 μL is a robust injection volume. It was shown that dilution of samples up to 10 times had no impact on the quantification of tasquinimod.

3.2.4. Stability of spiked samples

No degradation of tasquinimod was observed in spiked plasma neither at storage at room temperature for 6 h, nor during three freeze/thaw cycles. Also, tasquinimod was stable in processed spiked plasma samples when stored in the autosampler for up to 24 h at ambient temperature. Tasquinimod in spiked plasma was shown to be stable at least for 30 months of storage at −20 °C at all investigated concentrations in the interval 1–2400 nM (Fig. 4).

3.2.5. Incurred sample re-analysis

For the ISR study in CRPC patients, the average re-analysis result was 109% of the initial result, with only a single value outside the acceptance criteria ±20%. In the ISS investigation of healthy volunteers, the average obtained result at re-analysis was 104% of the initial result with only one re-analysis outside ±20%. Thus, the

Table 1
The repeatability (intra-assay precision), reproducibility (inter-assay precision) and overall accuracy bias for the determination of tasquinimod in plasma. The calculations were based on three batches of QC samples at four concentration levels in replicates of five.

Parameter	QC_LLOQ (1.0 nmol/L)	QC_L (2.5 nmol/L)	QC_M (70 nmol/L)	QC_H (2000 nmol/L)
Repeatability (%)	7.1	3.8	4.2	1.5
Reproducibility (%)	7.4	7.8	4.9	3.5
Overall bias (%)	4.7	2.3	3.2	1.3

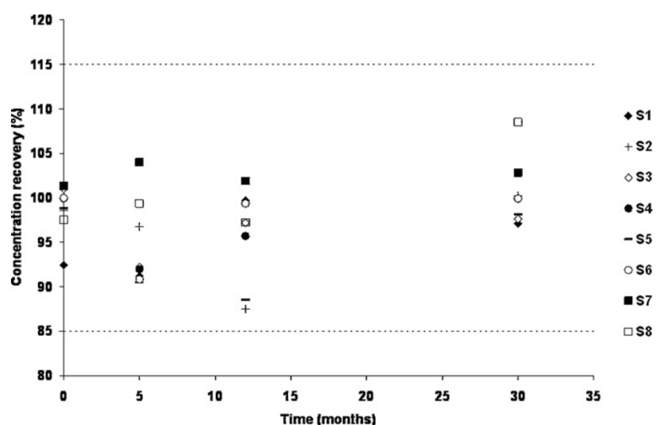

Fig. 4. Long-term stability of tasquinimod in spiked human plasma at −20 °C, studied up to 30 months for calibration samples S1–S8.

results in both studies well fulfilled the acceptance criteria for ISR. The results of the ISS investigation are shown in Fig. 5.

3.3. Clinical application

The results from the application of the validated method in a study of healthy volunteers are shown in Fig. 6. The time–concentration profiles obtained formed the basis for a successful pharmacokinetic profiling of tasquinimod in healthy volunteers.

4. Discussion

This paper describes the development, validation and application of a method for the determination and monitoring of tasquinimod in human plasma. This is the first bioanalytical method for this analyte described in the literature. The use of LC–MS/MS supported with a stable isotope labeled internal standard was found to be a successful approach for the quantification of tasquinimod in plasma.

In the development of sensitive LC–MS/MS methods, control of the carry-over effects in relation to the intended LLOQ is essential for reliable method performance. Apart from, robust procedures

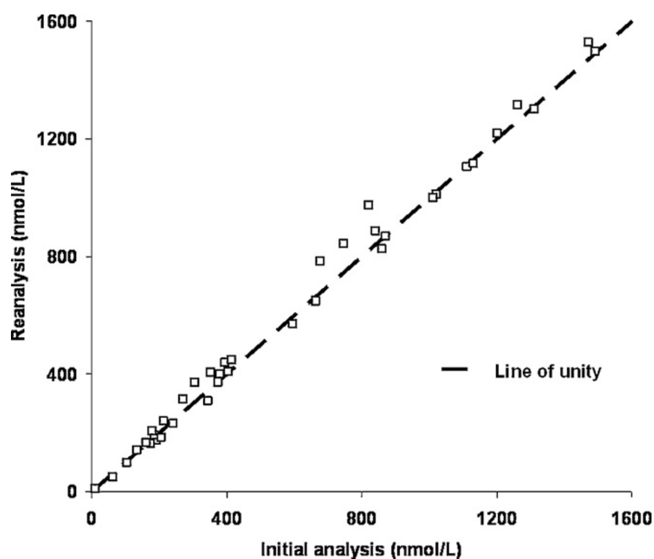

Fig. 5. Incurred sample stability (ISS) at −70 °C of tasquinimod in plasma from healthy volunteers. The re-analysis was performed 24 months after the initial analysis.

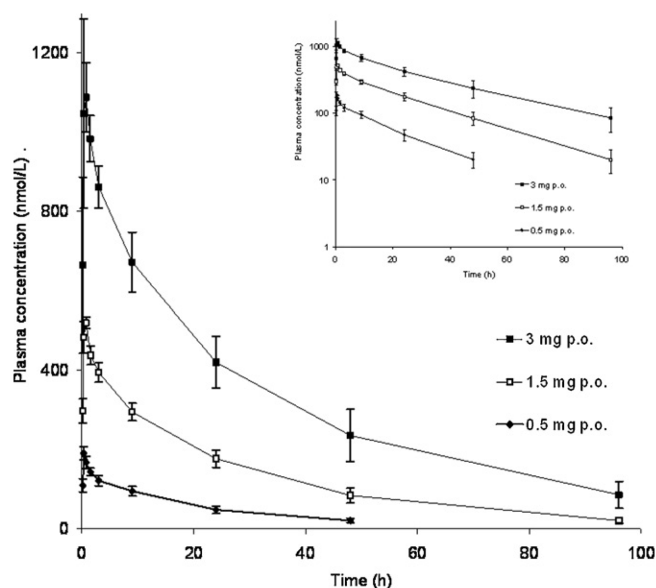

Fig. 6. Plasma concentration–time profiles for tasquinimod after administration of 0.5, 1.5 or 3.0 mg oral single dose to healthy volunteers. Each dose group consisted of four subjects. The inserted minor graph shows the logarithmic scale profiles.

for the washing of the analytical system, both spiked and study samples should be ordered in the run with respect to the expected analyte concentration. If necessary, injections of blank samples should be performed subsequent to samples with high concentrations. In our method, a robust injection port washing program was developed, based on the chemical properties of the analyte and of course also on consideration of the plasma matrix and the composition of the mobile phase. Tasquinimod is a weak acid with a pK_a of 6.5, and therefore the first wash step was conducted with a strong alkaline aqueous solution, enhancing the solubilization of the anionic form of the analyte. The second and final wash step with acetonitrile with 0.2% TFA was chosen for general washing out of lipophilic components and for compatibility with the pH of the subsequent mobile phase elution.

A main problem for the combination of LC and electrospray ionization techniques is signal variability due to ion suppression and/or enhancement. By introducing a stable isotope labeled IS which co-elutes with the analyte, the response of the analyte in the mass spectrometer is normalized. In other words, this kind of IS can compensate for instrumental, analytical and matrix variations, because the analyte and the IS are subjected to identical ion suppression. Therefore, minor matrix effects are expected in our developed method. This is demonstrated by the excellent robustness of our method. Furthermore, we have compared spiked calibration curves in human plasma vs. deionized water. A slight tendency of signal enhancement in plasma was observed, but no significant difference in analyte/IS ratio for the calibration curves in the two matrices were observed (unpublished results).

The LLOQ (1 nmol/L) and ULOQ (2400 nmol/L) of our method were established based on the anticipated concentration range, calculated from preclinical studies and human in vitro data. It was found that the established range was sufficient for determining concentrations at the late elimination phase as well as at the peak concentration without dilution, thus enabling robust pharmacokinetic calculations. The concentrations of the calibration samples were evenly distributed within the calibration range, although the guidelines allow any option. The same method was used in preclinical toxicological studies in mouse, rat and dog. The higher concentration of tasquinimod in plasma in this kind of studies could be managed using an injector valve with an internal injection loop

of 0.5 μL, i.e. 20 times lower than the validated method for human samples. This enables calibration at a concentration range 20 times higher, as the same amount of analyte is injected on-column in both cases (unpublished results).

The stability of the analyte in plasma was investigated in various ways during our validations. Investigation of the bench-top stability, freeze-thaw stability and long-term stability in freezer that tasquinimod is stable in plasma for a relevant period of time with respect to analysis. For the long-term stability we used a non-conventional approach by analyzing previous batches of calibration samples with different ages at one single occasion. The advantage of this approach is that the analytical day-to-day variation is eliminated from the data. The disadvantage is that no stability data are obtained during the study. Prior to the clinical studies and the 30 month long-term stability evaluation, stability of tasquinimod in human plasma had been demonstrated at −20 °C for up to 7 months using a similar experimental approach as the one described above, however with a single point measurement (unpublished results).

In recent years, additional regulatory demands on investigations regarding the stability of the analyte in plasma from dosed subjects (i.e. incurred samples) have emerged [13]. The scientific rationale for ISR is two-fold. Firstly, the incurred plasma matrix potentially may contain labile metabolites that may be back-converted to parent during sample storage, handling, processing and analysis. Secondly, ISR is used as a process control methodology to monitor the robustness of the bioanalytical method. Both our ISR and the ISS investigation described in this study well fulfill the proposed ISR acceptance criteria [12,13], allowing 1/3 of samples deviate >20%. It should be mentioned that these proposed ISR acceptance criteria differ in how to calculate the % deviation. We have adopted the EBF recommendation, since in which we believe the validity of the initial (i.e. reported) value is more correctly investigated. In addition we have performed three other ISR studies for tasquinimod, which also fulfill these criteria (unpublished results). Furthermore, we also performed an ISS study, investigating the long-term stability of tasquinimod in incurred samples. After 24 months of storage, no significant concentration deviations were observed and the results fulfilled the ISR criteria. Thus, we have demonstrated that tasquinimod is stable in incurred plasma, and that the performance of our method is robust in delivering reliable bioanalytical data. Actually, prior to human studies the bench-top short-term stability (0–6 h) as well as the autosampler storage stability for processed samples was studied in preclinical incurred samples. These additional parameters were studied during method development in order to evaluate the mentioned potential *ex vivo* decomposition of labile tasquinimod metabolites in incurred samples. No such metabolite decomposition was observed (unpublished results). This approach is not covered in proposed ISR guidelines, but may be considered to be of significant relevance.

Summarizing our stability investigations, it has been unambiguously shown that tasquinimod is stable in plasma under all investigated conditions, that there are no issues on metabolite decomposition affecting the quantified tasquinimod plasma concentrations, and that our developed method is convincingly robust.

The clinical development program for tasquinimod was initiated with a micro-dose study, in which four healthy volunteers were administered a single dose of 25 μg. For this study, a bioanalytical method based on solid phase extraction was developed with an LLOQ of 0.04 nmol/L (unpublished results). However, in retrospect the method described herein can be said to be sufficient for monitoring and evaluating PK even at this low dose level. In the continuation of the tasquinimod clinical development program, our method has been extensively used for support in pharmacokinetic and pharmacodynamic evaluations. At present, samples from 64 healthy volunteers and 248 CRPC patients have been successfully analyzed and, hitherto no analytical batches have failed due to unfilled QC samples criteria. Furthermore, the LC–MS/MS chromatograms of all analyzed pre-dose samples ($n = 312$) showed no interfering peaks, demonstrating excellent selectivity of the method.

In conclusion, we have developed and validated a sensitive, robust and selective method for the determination of tasquinimod in human plasma that has been successfully applied in several clinical trials.

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