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Quantification of KRAS inhibitor sotorasib in mouse plasma and tissue homogenates using liquid chromatography-tandem mass spectrometry

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ABSTRACT

Sotorasib is a KRAS inhibitor with promising anticancer activity in phase I clinical studies. This compound is currently under further clinical evaluation as monotherapy and combination therapy against solid tumors. In this study, a liquid chromatography-tandem mass spectrometric method to quantify sotorasib in mouse plasma and eight tissue-related matrices (brain, liver, spleen, kidney, small intestine, small intestine content, lung, and testis homogenates) was developed and validated. Protein precipitation using acetonitrile was utilized in 96-well format to extract sotorasib and erlotinib (internal standard) from mouse plasma and tissue homogenates. Separation of the analytes was performed on an Acquity UPLC® BEH C18 column by gradient elution of methanol and 0.1% formic acid in water at a flow rate of 0.6 ml/min. Sotorasib was detected by a triple quadrupole mass spectrometer with positive electrospray ionization in selected reaction monitoring mode. A linear calibration range of 2–2,000 ng/ml of sotorasib was achieved during the validation. Accuracy values were in the range of 90.7–111.4%, and precision values (intra- and interday) were between 1.7% and 9.2% for all tested levels in all investigated matrices. The method was successfully applied to investigate the plasma pharmacokinetics and tissue accumulation of sotorasib in female wild-type mice.

1. Introduction

Kirsten rat sarcoma virus oncogene (KRAS) mutations are often found in solid tumors such as lung carcinoma, pancreatic carcinoma, and colorectal carcinoma [1]. Non-small cell lung carcinoma (NSCLC) patients with a KRAS mutation have been associated with poor prognosis [2,3], making KRAS a promising target for intervention in cancer therapy. However, KRAS was long known as an "undruggable" [1] until sotorasib (AMG510, Fig. 1) entered the clinical stage in 2019 [4].

Sotorasib showed a promising anticancer activity in its early discovery stage. It is believed that the acrylamide group of sotorasib responsible for the KRAS inhibition by covalently bound to $KRAS^{G12C}$ [5]. A phase I dose-escalation clinical study reported a promising anticancer activity of sotorasib in NSCLC patients but a less pronounced positive result for colorectal cancer patients [6]. Therefore, further clinical evaluation of sotorasib as monotherapy or in combination with various other anticancer agents in a number of solid tumors is ongoing [6].

Abbreviations: AUC, Area Under Curve; BEH, Bridged Ethyl Hybrid; BSA, Bovine Serum Albumin; CRC, Colorectal Carcinoma; DLT, Dose Limiting Toxicity; EMA, European Medicine Agency; FVB, Friend Virus B-Type; IS, Internal standard; KRAS, Kirsten Rat Sarcoma; LC, Liquid Chromatography; LC-MS/MS, Liquid Chromatography tandem Mass Spectrometry; LLE, Liquid – liquid extraction; LLoQ, Lower Limit of Quantification; MF, Matrix Factor; MS, Mass Spectrometry; NSCCL, Non-small Cell Lung Carcinoma; PP, Protein precipitation; QC, Quality Control; RAS, Rat Sarcoma; TCKI, Targeted Covalent Kinase Inhibitor; US FDA, United States Food and Drug Agency.

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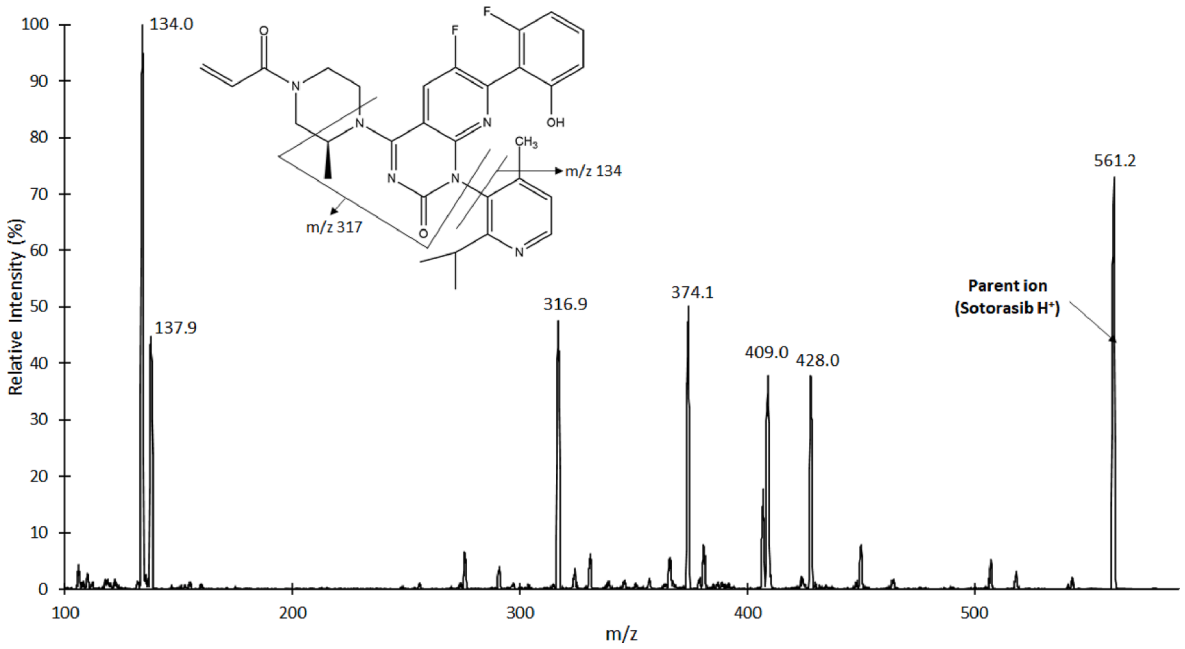

Fig. 1. Chemical structure of sotorasib and the product ion mass spectrum formed by collision-induced dissociation of protonated sotorasib (*m/z* 561.2 @ 49 V) with the expected fragment.

Cancer pharmacotherapy is characterized by relatively narrow therapeutic windows and is often subject to individual dose adjustment considering each patient's conditions to ensure its safety-efficacy balance [7,8]. Thus, more insight into sotorasib drug levels is essential. Pharmacokinetic profiles in both preclinical and clinical experiments are also critical to tailor future clinical application [9] of this promising drug. A reliable quantitative determination of sotorasib in biological samples is essential and should first be established to obtain such knowledge. To date, there is only one paper describing a validated method of sotorasib in mouse plasma matrix but not in mouse tissues [10]. The described paper utilized LC-MS/MS and liquid–liquid extraction (LLE) as the sample pretreatment method [10]. However, LLE is more cumbersome and more time-consuming than simple protein precipitation (PP) due to the necessity of extract evaporation and reconstitution [11].

Therefore, in this study, we developed and validated a new LC-MS/MS method to quantify sotorasib in mouse plasma, seven tissue and small intestine content homogenates with PP as sample pretreatment. This developed method can support further preclinical and clinical investigation of sotorasib to obtain more insight into the pharmacokinetics and tissue distribution of this drug.

2. Material & methods

2.1. Chemicals and reagents

Sotorasib (>98%, M_w = 560.59 g/mol) was supplied by Carbosynth (Compton, Berkshire, UK). Erlotinib (>99%, as hydrochloric acid, M_w = 393,44 g/mol) used as internal standard (IS) was obtained from the same company under its previous name Sequoia Research Products (Pangbourne, UK). Methanol (HPLC grade) and water (ULC-MS grade) were acquired from Biosolve (Valkenswaard, The Netherlands). Analytical grade formic acid was supplied by Merck KGaA (Darmstadt, Germany). Pooled CD-1 mouse lithium heparin plasma (female) and human lithium heparin plasma (mixed gender) were obtained from Sera Laboratories (West Sussex, UK).

2.2. Tissue homogenization

The blank mouse tissue homogenates were prepared by mixing the whole (weighed) organ with 2% (w/v) of bovine serum albumin (BSA) in milli-Q water on an iced-condition [12,13]. A FastPrep-24™ 5G instrument (M.P. Biomedicals, Santa Ana, USA) was utilized for this purpose. The volumes of BSA used were 1 ml for brain, 3 ml for liver, 2 ml for kidney, 1 ml for spleen, 3 ml for small intestine, 2 ml for small intestine content, 1 ml for testis, and 1 ml for lung.

2.3. Analytical instruments

A chromatographic system of Shimadzu Nexera X2 (Kyoto, Japan) equipped with a DGU-250 AR degasser, two LC30-AD pumps, a Sil30-ACmp autosampler, and a CTO-20 AC column oven was utilized for the separation. For the detection, a triple quadrupole mass spectrometer AB-SCIEX QTRAP® 5500 (Ontario, Canada), equipped with a Turbo Ion V™ TurboIonSpray® probe and an inlet valve were used. Analyst 1.6.2. software (Sciex) was used for instrument control and data collection. MultiQuant 3.0.1. software (Sciex) was used to process all LC-MS/MS data. A preliminary identification check of sotorasib was performed with a high-resolution time-of-flight mass spectrometer AB-SCIEX Triple TOF® (Ontario, Canada).

2.4. LC-MS/MS conditions

The injection (2 µl by partial loop filling) was applied on an Acquity UPLC® BEH C18 column (30 X 2.1 mm, d_p = 1.7 µm, Waters, Milford, MA, USA) protected by UPLC® BEH C18 Vanguard pre-column (5 × 2.1 mm, d_p = 1.7 µm, Waters), and prefilter (2.1 mm, 0.2 µm, Waters). The column and autosampler temperature was maintained at 40 °C and 4 °C, respectively during the analytical run. The gradient elution was performed at 0.6 ml/min with 0.1% (v/v) formic acid in water (A) and methanol (B). After the injection, the percentage of methanol increased linearly from 45% to 65% in 1.0 min, followed by flushing with 100% methanol for 0.3 min. Finally, the column was reconditioned at the initial condition to 45% B for 0.6 min. The eluent was transferred to the ionization interface by the divert valve from 0.5 min to 1.3 min after the

Table 1
Individual SRM parameters of sotorasib and erlotinib.

Compound	*m/z* (Q1)	*m/z* (Q3)	DP (V)	CE (V)	CXP (V)
Sotorasib	561.2	134.0*	71	49	8
		317.1	71	57	18
Erlotinib	394.1	278.1*	50	47	12

DP: declustering potential, CE: Collision energy, CXP: Collision cell exit potential.
* used for quantification.

injection.

Selected reaction monitoring (SRM) in positive mode was used as the detection mode with parameters: curtain gas 15 psi, ion spray voltage 1,400 V, temperature 700 °C, ion source gas (1) 60 psi, ion source gas (2) 75 psi. These optimum parameters were obtained by direct introduction of 1000 ng/ml sotorasib at 5 μl/min in methanol/0.1% formic acid in water (50:50 v/v) into the mass spectrometer. The individual SRM parameters of both sotorasib and erlotinib (IS) are listed in Table 1.

2.5. Stock & working solutions

A 500,000 ng/ml sotorasib stock solution was prepared by weighing 200–400 μg of sotorasib and dissolving it in methanol. A 50,000 ng/ml sotorasib working solution was obtained by diluting the stock solution 10-fold with 50% methanol (v/v) in water. This solution was used further to prepare calibration samples in mouse plasma. A second set of stock and working solutions with the same level were also prepared and used further to make quality control (QC) samples. A 1,000 μg/ml erlotinib stock solution was prepared by weighing 500–700 μg of erlotinib and dissolving it in methanol. A working solution of 100,000 ng/ml erlotinib was obtained by diluting the stock solution 10-fold with 50% methanol in water (v/v). Further, a serial dilution of erlotinib working solution was performed to obtain 100 ng/ml and 10 ng/ml of erlotinib in acetonitrile. The 10 ng/ml erlotinib was used as a daily IS. All solutions were stored at −30 °C.

2.6. Calibration and quality control samples

The highest calibration sample was prepared by diluting 50,000 ng/ml of sotorasib working solution to 2,000 ng/ml in blank pooled mouse plasma in a polypropylene tube. Until further use, this sample was stored at −30 °C. The highest calibration sample was further diluted for daily calibration to 1,000, 200, 100, 20, 10, and 2 ng/ml in blank pooled mouse plasma. QC samples were prepared from the second 50,000 ng/ml sotorasib working solution by sequential dilution to 1,600 (high), 80 (medium), 4 (low), and 2 ng/ml (lower limit of quantification / LLoQ) in blank pooled mouse plasma. QC samples at 80 ng/ml (medium) were also prepared in pooled mouse tissue homogenates of brain, liver, kidney, spleen, small intestine, small intestine content, lung, and testis. QC samples at 16,000 and 8,000 ng/ml in pooled mouse plasma were also prepared by a direct dilution from the second stock solution.

2.7. Sample preparation

Ten μl of plasma or tissue homogenates were pipetted into a polypropylene 96-well plate with 200 μl conical bottom wells and 20 μl of 10 ng/ml erlotinib in acetonitrile was added. The 96-well plates were closed with silicone mats and shaken briefly by a vortex mixer. Then, the plate was centrifuged at 3,500 × g for 5 min, and 20 μl supernatant was transferred into a 96-deep well plate with 1 ml round bottom wells. Subsequently, 200 μl of 25% methanol (v/v) in water was added into the supernatant, followed by gently mixing the plate. The prepared plated was then put in the autosampler and was ready for injection.

2.8. Analytical method validation

Guidelines on bioanalytical method validation from the United States Food & Drug Administration (US FDA) [14] and European Medicine Agency (EMA) [15] were used as the bioanalytical validation framework for the new assay. The mouse plasma matrix was fully validated according to those guidelines, while the tissue homogenates were partially validated in the range of 2–2,000 ng/ml of sotorasib.

2.8.1. Calibration

All calibration samples were prepared in duplicate for each daily use along with additional blank (no analyte) and double blank (no analyte, no IS) samples (n = 18). Least square linear regression with the reversed square of the concentration ($1/x^2$) as the weighting factor was used to define the calibration curve using the ratio of the analyte (sotorasib) and the IS (erlotinib) peak areas.

2.8.2. Precision & accuracy

The QC-high (1,600 ng/ml), -medium (80 ng/ml), -low (4 ng/ml), and -LLoQ (2 ng/ml) in pooled mouse plasma and -medium (80 ng/ml) in eight tissue homogenates were used to access the precision and accuracy. Precision (both intra- and inter-day) and accuracy were performed in sextuple analysis for each QC level on three separate days (n = 18 per QC). In addition, dilution integrity was investigated by diluting 8,000 and 16,000 ng/ml of sotorasib in mouse plasma to QC-high levels in human lithium heparin plasma with 5-fold and 10-fold dilution factor, respectively (n = 6 for every dilution factor).

2.8.3. Selectivity

To assess the selectivity of the assay, six individual lithium heparin mouse plasma and 32 individual tissue homogenates (4 individual samples for each tissue) were processed. Each sample was analyzed as LLoQ spiked (2 ng/ml sotorasib) and double blank (no analyte, no IS) sample.

2.8.4. Recovery and matrix effect

Three types of samples in pooled mouse plasma at three different QC levels, QC-high, -medium, and -low, in four replications for each level were prepared to assess the recovery and matrix effect of the assay. The first sample was normal plasma sample (A) treated as stated by sample preparation step (section 2.7). A similar sample as A with the analyte added after extraction (B) and samples without any matrix constituent (C) were the second and third samples, respectively. Recovery was calculated from ratio A/B, while matrix effect was determined by the ratio B/C.

The relative matrix effect was assessed using the same samples used to evaluate selectivity: 6 individual plasma and 32 individual tissue homogenates. These samples were prepared at QC-high and -low levels. The relative matrix effect was calculated by comparing their responses to the reference solutions without the presence of any matrix at the same level.

2.8.5. Stability

The stability of sotorasib was investigated in the QC-high and -low mouse plasma samples. Quadruplicate analysis of these samples was performed after exposure to room temperature (ca. 22 °C) for 6 h, storage at −30 °C interrupted by four freeze–thaw cycles (thawing at room temperature for ± 1 h, and freezing again at least for 20 h), and after three months storage at −30 °C. Additional room temperature stability for both QC levels was determined in mouse plasma after 2, 4, 6, 8, 16, and 24 h exposure to ambient temperature. Stability of sotorasib in pooled tissue homogenates (brain, liver, kidney, spleen, small intestine, lung, and testis) and pooled small intestine content homogenate was assessed at the QC-medium level after exposure to room temperature for 6 h.

The stability of sotorasib in stock solutions (in methanol) and

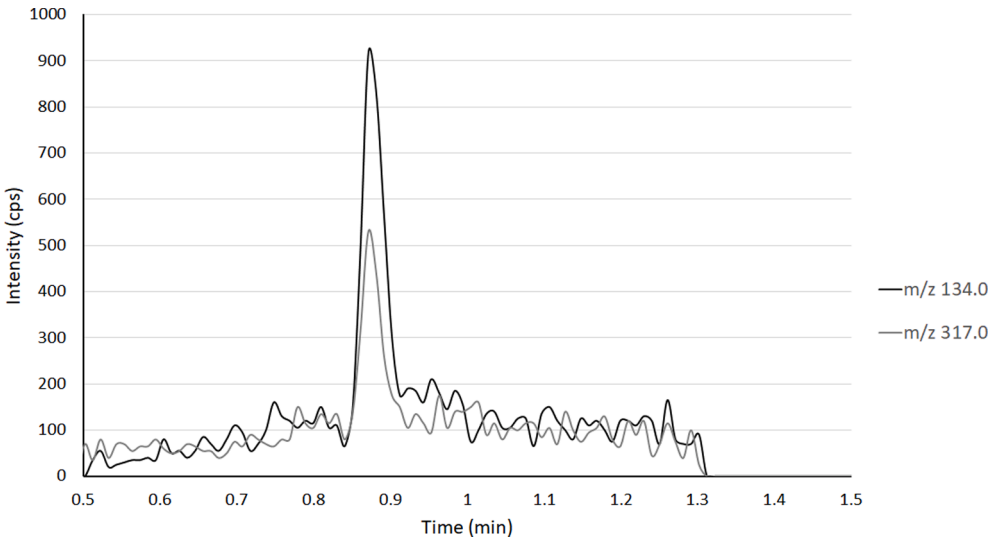

Fig. 2. Response of sotorasib in plasma sample spiked with 2 ng/ml of sotorasib in both mass transitions (*m/z* 516.2 → 134.0 and 516.2 → 317.0).

working solutions (in 50% v/v methanol) was tested. These solutions were measured after exposure to room temperature with the presence of light for 6 h and after storage at −30 °C for three months. The response of these solutions was compared to the freshly prepared solutions at the same level.

An analytical run with 24 QC samples (same samples as accuracy and precision) was reinjected and reanalyzed after 2 weeks of storage at 4 °C in the autosampler.

2.8.6. *Incurred sample reanalysis*

Reanalysis of sixty-six samples from preclinical study (n = 24 for plasma, n = 6 for each investigated tissue-related matrix) was performed one month after the first analysis.

2.9. *Pharmacokinetic study in mice*

2.9.1. *Mouse treatment*

The pharmacokinetic study used six female wild-type (FVB/NRj genetic background) mice with ages between 8 and 16 weeks. The mice were orally administered 20 mg/kg bodyweight of sotorasib after they were fasted for 2–3 h. Subsequently, their blood was withdrawn from the tail vein at 0.125, 0.25, 0.5, 1, and 2 h after drug administration. Four hours after drug administration, the mice were anesthetized with isoflurane to collect the final blood samples by cardiac puncture. Finally, the mice were sacrificed by cervical dislocation, and the tissues of interest (brain, liver, kidney, spleen, small intestine, and lungs) were immediately harvested. Small intestine content was also collected during organ harvesting. The housing and handling of the mice followed the institutional guidelines of the Netherlands Cancer Institute and complied with the Dutch and EU legislation.

Plasma samples were acquired from blood samples by centrifugation at 9,000*g* for 6 min at 4 °C. Small intestine and lung were rinsed with saline before homogenization with 2% (w/v) bovine serum albumin (BSA). All samples were stored at – 30 °C before further analysis. All the plasma samples were diluted 5 times, while small intestine and small intestine contents were diluted 10 times with human lithium heparin plasma before quantitative analysis. The rest of biological matrices can be pretreated directly according to section 2.7.

2.9.2. *Pharmacokinetic calculations*

Pharmacokinetic parameters were reported as mean ± standard deviation. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly calculated from the highest concentration and its correlative time points. The terminal half-life ($T_{1/2}$) was calculated from $C_{0.25}$ to C_4. The area under the plasma concentration–time curve ($AUC_{0\rightarrow 4}$ & $AUC_{0\rightarrow\infty}$) was calculated using the trapezoidal rule. Sotorasib concentrations in tissue and small intestine content were calculated based on their individual weight. All the pharmacokinetic parameters mentioned were calculated manually with MS Excel.

3. Results and discussion

According to our knowledge, this method is the first validated bioanalytical method for sotorasib in mouse plasma and several mouse tissue homogenates to date. The presented bioanalytical method of sotorasib is efficient with a two-minute run time supported by a straightforward sample pretreatment procedure. Moreover, this method is capable of analyzing a small volume (10 µl) of samples. Therefore, this bioanalytical method is suitable to support preclinical studies of sotorasib, as demonstrated here.

3.1. *Method development*

Electrospray ionization in positive mode was optimized for a single protonated molecule of sotorasib (*m/z* = 561.2) to obtain a maximum response. Fig. 1 shows a product spectrum of a single protonated sotorasib obtained by triple quadrupole mass spectrometry. A high-resolution product spectrum of single protonated sotorasib and proposed dissociation pathways is provided as supplementary information. The product masses optimized in the development stage were *m/z* 134.0 and 317.0. However, only product mass *m/z* 134.0 was used to quantify sotorasib due to its higher intensity and similar noise background compared to product mass of *m/z* 317.0 (Fig. 2). Therefore, the *m/z* 561.2 → 134.0 mass transition provides a higher sensitivity than 561.2 → 317.0.

The chromatographic separation was optimized and developed on the Acquity UPLC™ BEH C18 column based on MS response, peak shape, and retention time. The acidic condition with 0.1% formic acid is preferred for the chromatographic separation compared to alkaline conditions. Despite sotorasib having a lower response than under alkaline condition (0.2% ammonium hydroxide in water), the acidic condition offered a better stability of the analyte's retention time. We observed a retention time shift of *ca.* 0.5 min over several days when ammonium hydroxide was used as a mobile phase. The CO_2 absorption into ammonium hydroxide solution may cause a decrease of pH of the

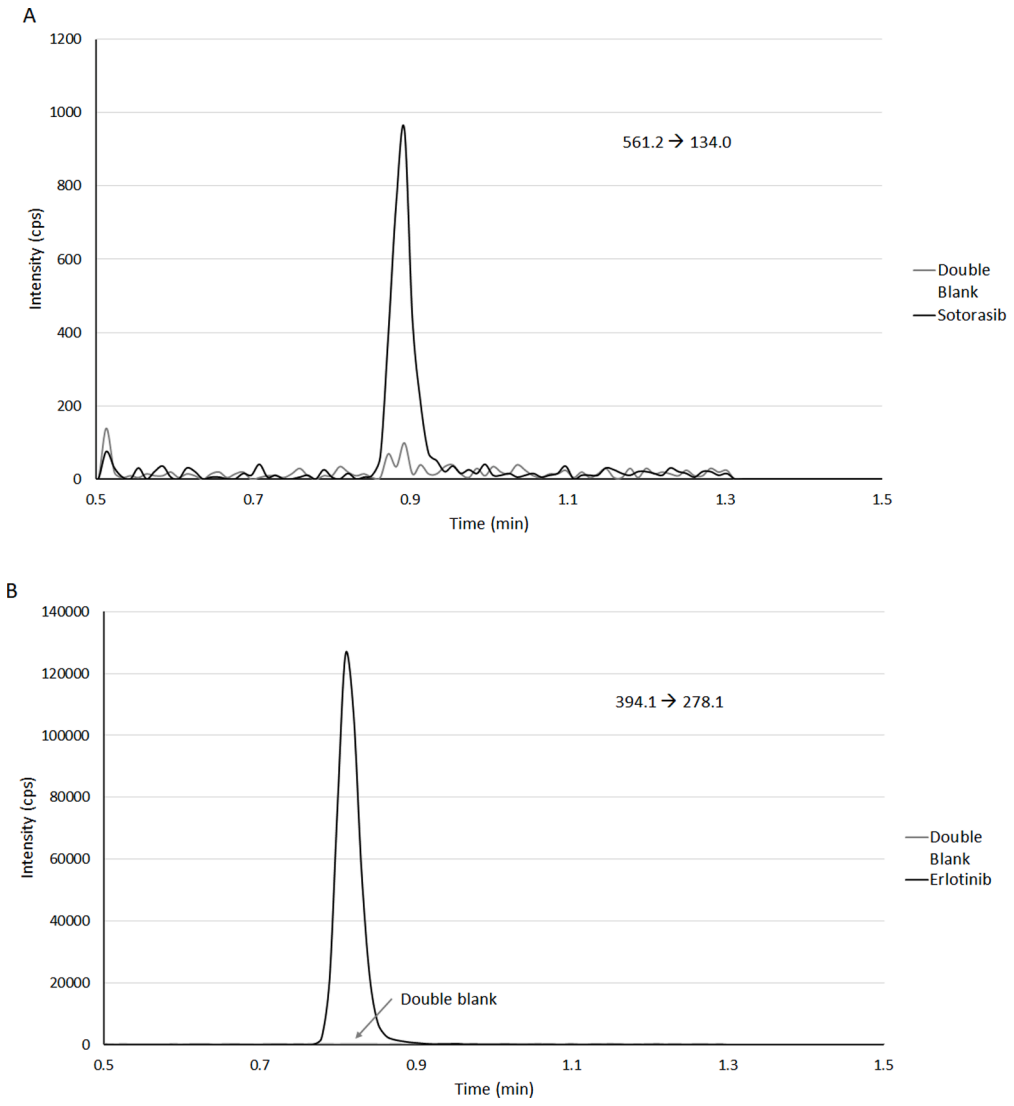

Fig. 3. Chromatogram of sotorasib (A) and erlotinib (B) in mouse plasma at the LLoQ level of sotorasib (2 ng/ml) compared to double blank plasma sample (in grey).

eluent to below 9 [16], possibly leading to a retention time shift of sotorasib which has a predicted pK_a value of 7. Although sotorasib has a lower response in acidic condition, the developed method was proven capable of achieving an excellent sensitivity to quantify sotorasib down to 2 ng/ml in all tested biological matrices.

Further, methanol was utilized as the organic modifier since it gave a higher theoretical plate number than acetonitrile when used in the mobile phase. Due to the unavailability of a stable labeled-isotope compound of sotorasib, several compounds were investigated as an IS during the method development. Erlotinib was selected as the IS in this assay after screening several compounds possessing similar masses and chromatographic properties as sotorasib. Erlotinib was monitored at *m/z* 394.1 → 278.1, adopting the previous method using the same instrument [17]. The final combination of water, formic acid, and methanol as mobile phase showed satisfactory chromatographic properties of sotorasib and erlotinib with a total of 1.9 run time. This mobile phase eluted both compounds on the used column within 1 min, as depicted by Fig. 3.

Sample preparation developed for this method was protein precipitation utilizing acetonitrile as the organic solvent. Protein precipitation is more straightforward than other methods, i.e., solid-phase extraction and liquid–liquid extraction, as used in the existing method of Madhyastha *et al.* [10]. Applying this method in a 96-well plate offers an enhanced sample throughput with hundreds of samples that can be executed in a shorter time compared to conducting this technique in an individual Eppendorf tube. Protein precipitation with acetonitrile is the most often used method to treat targeted covalent kinase inhibitors, characterized by acrylamide moiety as a neutrophilic warhead, in biological matrices [11]. Since sotorasib also possesses an acrylamide moiety, protein precipitation may be effective to extract this compound in a variety of biological matrices. Further, this decision was confirmed by the recovery percentage of sotorasib in a variety of biological matrices (section 3.2.4.).

Table 2
Assay performance data (n = 18; 3 days) of sotorasib in mouse plasma.

QC Level	Concentration (ng/ml)	Accuracy (%)	Intraday precision (%)	Interday precision (%)
High (H)	1600	90.7	1.7	2.1
Medium (M)	80	98.3	4.4	4.6
Low (L)	4	96.3	3.8	4.9
Lower Limit of Quantification (LLoQ)	2	97.8	8.0	9.2

Table 3
The assay performance and stability (measured as % recovery after 6 h exposure at room temperature) of 80 ng/ml sotorasib in 7 tissue and small intestine content homogenates.

Tissue	Accuracy (%)	Intraday precision (%)	Interday precision (%)	6 h RT stability
Brain	101.8	3.2	4.3	98.5 ± 4.1
Liver	100.2	2.9	3.0	101.6 ± 4.6
Kidney	100.2	4.3	8.4	93.7 ± 6.2
Spleen	100.1	3.6	4.8	88.4 ± 1.4
Small intestine	111.4	7.5	8.5	91.8 ± 3.1
Small intestine content	106.1	5.3	6.7	92.6 ± 4.5
Lung	111.0	2.2	5.2	96.1 ± 4.1
Testis	95.9	4.7	7.9	89.9 ± 2.5

h = hour, RT = room temperature.

3.2. Validation

3.2.1. Calibration

The relative response of sotorasib showed a linear trend over the investigated range of 2–2,000 ng/ml. Thus, linear regression equation (Y = aX + b) was used for calibration results. Parameter a is the slope, b denotes the intercept, Y being the area ratio of sotorasib to erlotinib, X is the concentration of sotorasib in ng/ml, and R^2 determines the regression coefficient. The average equation of the calibration curve for eight consecutive runs (reported as mean ± SD) was Y = 30.8 (±2.6)·10^{-4} X + 7.6 (±7.7)·10^{-4} with R^2 = 0.9988 (±0.0007).

3.2.2. Precision & accuracy

The performance of the developed analytical method was reported as accuracy and precision data at four QC levels for pooled mouse plasma (Table 2) and at the QC-medium level for each investigated tissue and small intestine content homogenate (Table 3). The accuracy, intra-day precision, and inter-day precision for QC-high, QC-medium, QC-low in plasma were within 15% and for QC-LLoQ within 20%. Moreover, the accuracy and precision for QC-med in all tested tissue-related matrices were within ± 15%. These results fulfilled the guideline requirements used as the framework [14,15]. Dilution integrity in human plasma was obtained with an accuracy of 97.4% and precision of 4.1% for 5-fold dilution at 8,000 ng/ml; and an accuracy of 96.9% and precision of 2.2% for 10-fold dilution at 16,000 ng/ml, confirming the reliability of this dilution factor to be used for small volume samples as in a small rodent pharmacokinetic study.

3.2.3. Selectivity

None of the individual blank samples (6 plasma and 32 homogenates) were exceeding 20% of the sotorasib LLoQ response, and neither of the responses of erlotinib exceeding 5% of its normal response as required by the guidelines [14,15]. The measured sotorasib levels in LLoQ (2 ng/ml) spiked samples were 1.96 ± 0.18 ng/ml in plasma (n = 6) and 2.07 ± 0.21 ng/ml in tissue homogenates (n = 32), with only one liver homogenate sample exceeding 120% of the target value. These results indicate that the developed method was capable to quantify sotorasib down to 2 ng/ml.

3.2.4. Recovery & matrix effect

The extraction recovery data for QC-high, QC-med, QC-low and IS in pooled mouse plasma were 95.0 ± 4.8%, 90.0 ± 5.2%, 96.3 ± 8.4%, 92.6 ± 3.9% respectively. Further, the matrix effect data of pooled mouse plasma tested at three QC-levels were 95.0 ± 2.6%, 94.4 ± 1.4%, and 93.1 ± 6.4%. The IS-normalized matrix factor (MF) was calculated to assess the relative matrix effect of individual matrices [15]. The relative matrix effect of the individual matrix (n = 38 for each level) was 109.7 ± 3.8% for QC-high and 111.7 ± 9.0% for the QC-low level. The variations of calculated IS-normalized MF for QC-high were 4.7% in plasma matrices and ranged from 0.9 to 5.2% in tissue homogenates, whereas for QC-low samples variations were 9.5% for plasma and ranged from 0.9 to 8.9% in the investigated homogenates, as required by the guideline [15]. These results indicate that there is no significant interference of the investigated matrices in sotorasib quantification in this developed method.

Table 4
Stability data (reported as mean recovery (%) ± SD; n = 4) of sotorasib in mouse plasma.

Condition	QC-high	QC-low
6 h at room temperature	93.2 ± 2.8	89.0 ± 5.0
4 freeze–thaw cycles	90.6 ± 1.9	89.0 ± 3.8
3 months at −30 °C	108.0 ± 8.8	87.9 ± 2.5

3.2.5. Stability

The stability data of sotorasib in lithium heparin plasma at QC-high and -low levels are presented in Table 4, while the stability of sotorasib in variety tissue homogenates at the QC-medium level at room temperature for 6 h is given in Table 3.

Sotorasib is stable under all tested conditions during the analytical procedure (Table 4). However, at room temperature, we observed a low stability of sotorasib. Thus, we conducted a further investigation for the room temperature stability in plasma. Our data describe that sotorasib is stable up to 6 h at ambient temperature and a slightly under the 85% requirement when QC-high level was exposed up to 8 h at room temperature, as depicted by Fig. 4. This data shows a similarity with the instability of other targeted covalent kinase inhibitors (TCKIs). Most TCKIs, except for ibrutinib and osimertinib, which have shorter stability periods, are stable for *ca.* 6 h when exposed to room temperature [11].

This low stability may be caused by the reactive acrylamide group of sotorasib. It has been well documented that small molecule drugs possessing an acrylamide-derivative group, mostly in TCKIs, show low stability at room temperature [11]. The acrylamide group of sotorasib is an electrophilic warhead that can freely react with nucleophiles such as thiol and amine groups naturally present in biological matrices. Moreover, the acrylamide group of sotorasib is not sterically protected by another group. This configuration enables free nucleophiles to easily react with this moiety, possibly leading to the limited stability of this compound at ambient temperature.

3.2.6. Incurred sample reanalysis

Incurred sample reanalysis of mouse pilot study samples one month after initial analysis (n = 24 for plasma and 6 for each tissue-related matrices) resulted in 17 samples in a difference exceeding 20%. This fulfills the guideline requirements, which allows 33% of samples (<22 out of 66 samples) to have more than a 20% difference compared to the initial analysis [14,15].

3.3. Pharmacokinetic study in mice

After successful validation, the new method was used to investigate sotorasib concentration in plasma and tissues in a pilot study during 4 h after a single oral dose of 20 mg/kg bodyweight to female FVB/NRj genetic background mice (n = 6). Sotorasib shows a rapid absorption with C_{max} being achieved within 15 min after administration as depicted by Fig. 5. The obtained calculated pharmacokinetics parameters were: T_{max} = 0.21 ± 0.06 h, C_{max} = 4,231 ± 1,208 ng/ml, $T_{1/2}$ = 0.60 ± 0.06 h, $AUC_{0\rightarrow4}$ = 3,766 ± 896 ng.h.ml^{-1}, $AUC_{0\rightarrow\infty}$ = 53,811 ± 898 ng.h.ml^{-1}.

Fig. 6 illustrates the distribution of sotorasib in tissues and small intestine content. The sotorasib concentration in brain is much lower than in other tissues measured. This low concentration is likely caused

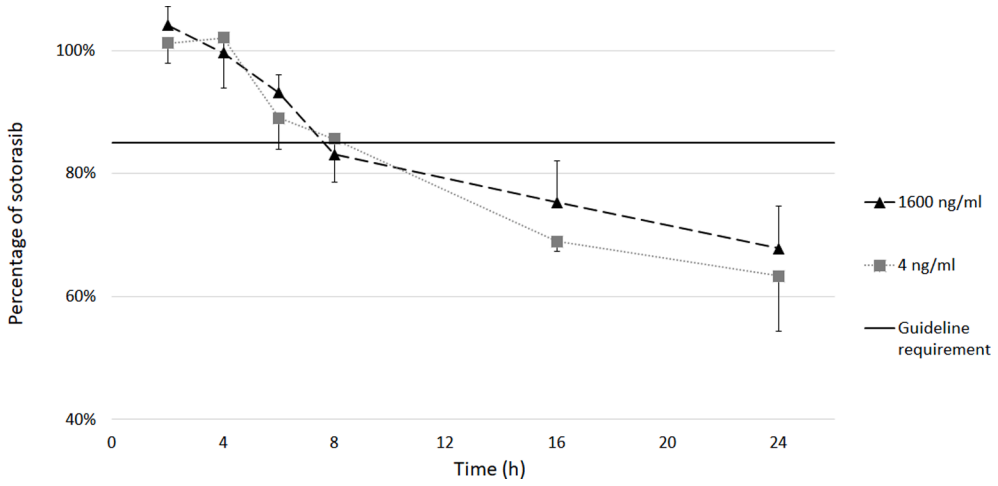

Fig. 4. Room temperature stability data (reported as the average of % recovery, n = 4) of sotorasib in mouse plasma.

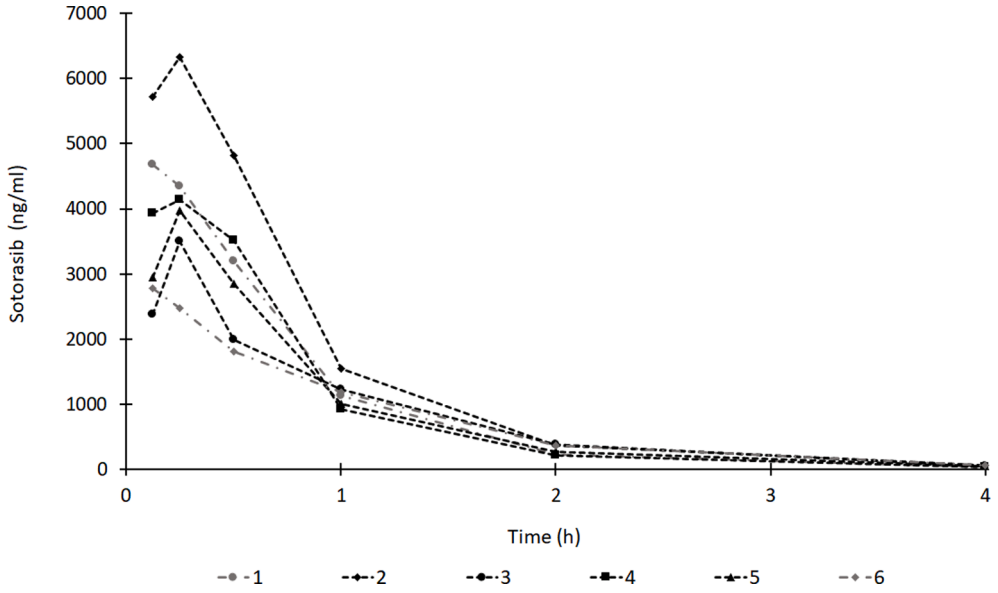

Fig. 5. Pharmacokinetic plot of six mice (FVB/NRj genetic background) treated orally with 20 mg/kg of sotorasib.

by the blood–brain barrier. In contrast, a very high concentration of sotorasib was found in small intestine content and small intestine. This result is possibly caused by the unabsorbed drug in the small intestine lumen or high transport of sotorasib into the gut. In order to establish a better insight into this matter, further mouse studies are necessary.

4. Conclusions

A bioanalytical method to quantify the first KRAS inhibitor sotorasib in plasma and tissues has been successfully developed and validated. The reported method was capable of analyzing 10-µl sample volumes with simple and efficient protein precipitation with acetonitrile in a 96-well format. The accuracy and precision at all levels in all bio-matrices tested fulfilled the requirement of international guidelines. The method showed neither a matrix effect nor any significant extraction loss. Lastly, this method successfully supported a pharmacokinetic and tissue distribution study in mice and thus can be used for further preclinical investigation of sotorasib.

CRediT authorship contribution statement

Irene A. Retmana: Methodology, Validation, Formal analysis, Investigation, Visualization, Writing - original draft. **Nancy H.C. Loos:** Formal analysis, Investigation, Visualization, Writing - review & editing. **Alfred H. Schinkel:** Conceptualization, Resources, Writing - review & editing, Supervision. **Jos H. Beijnen:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision. **Rolf W. Sparidans:** Conceptualization, Resources, Writing - review & editing, Supervision.

Declaration of Competing Interest

None.

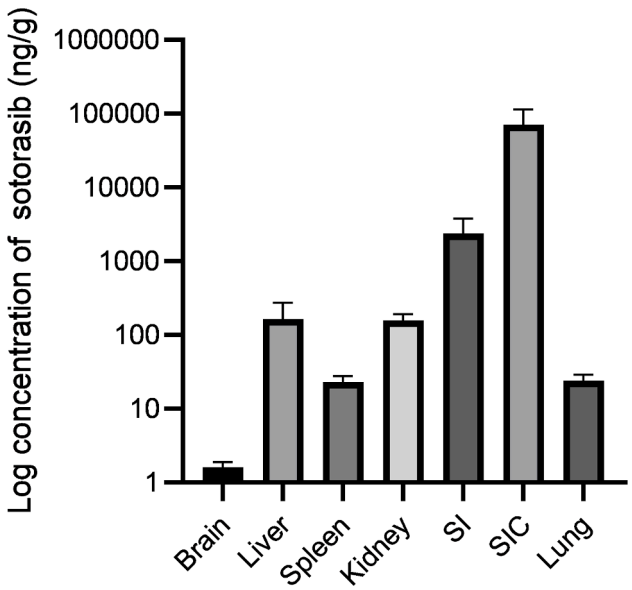

Fig. 6. Concentration of sotorasib in 6 tissues and small intestine content in FVB/NRj mice (n = 6), 4 h after oral administration of 20 mg/kg. SI: small intestine, SIC: small intestine content.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2021.122718.

References

[1] A.D. Cox, S.W. Fesik, A.C. Kimmelman, J. Luo, C.J. Der, Drugging the undruggable RAS: Mission Possible? Nat. Rev. Drug Discov. 13 (2014) 828–851, https://doi.org/10.1038/nrd4389.

[2] B. El Osta, M. Behera, S. Kim, L.D. Berry, G. Sica, R.N. Pillai, T.K. Owonikoko, M. G. Kris, B.E. Johnson, D.J. Kwiatkowski, L.M. Sholl, D.L. Aisner, P.A. Bunn, F. R. Khuri, S.S. Ramalingam, Characteristics and outcomes of patients with metastatic KRAS-mutant lung adenocarcinomas: The Lung Cancer Mutation Consortium Experience, J. Thorac. Oncol. 14 (2019) 876–889, https://doi.org/10.1016/j.jtho.2019.01.020.

[3] S.Y. Liu, H. Sun, J.Y. Zhou, G.L. Jie, Z. Xie, Y. Shao, X. Zhang, J.Y. Ye, C.X. Chen, X. C. Zhang, Q. Zhou, J.J. Yang, Y.L. Wu, Clinical characteristics and prognostic value of the KRAS G12C mutation in Chinese non-small cell lung cancer patients, Biomark. Res. 8 (2020) 22, https://doi.org/10.1186/s40364-020-00199-z.

[4] J. Canon, K. Rex, A.Y. Saiki, C. Mohr, K. Cooke, D. Bagal, K. Gaida, T. Holt, C. G. Knutson, N. Koppada, B.A. Lanman, J. Werner, A.S. Rapaport, T. San Miguel, R. Ortiz, T. Osgood, J.R. Sun, X. Zhu, J.D. McCarter, L.P. Volak, B.E. Houk, M. G. Fakih, B.H. O'Neil, T.J. Price, G.S. Falchook, J. Desai, J. Kuo, R. Govindan, D. S. Hong, W. Ouyang, H. Henary, T. Arvedson, V.J. Cee, J.R. Lipford, The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity, Nature 575 (2019) 217–223, https://doi.org/10.1038/s41586-019-1694-1.

[5] B.A. Lanman, J.R. Allen, J.G. Allen, A.K. Amegadzie, K.S. Ashton, S.K. Booker, J. J. Chen, N. Chen, M.J. Frohn, G. Goodman, D.J. Kopecky, L. Liu, P. Lopez, J. D. Low, V. Ma, A.E. Minatti, T.T. Nguyen, N. Nishimura, A.J. Pickrell, A.B. Reed, Y. Shin, A.C. Siegmund, N.A. Tamayo, C.M. Tegley, M.C. Walton, H.L. Wang, R. P. Wurz, M. Xue, K.C. Yang, P. Achanta, M.D. Bartberger, J. Canon, L.S. Hollis, J. D. McCarter, C. Mohr, K. Rex, A.Y. Saiki, T. San Miguel, L.P. Volak, K.H. Wang, D. A. Whittington, S.G. Zech, J.R. Lipford, V.J. Cee, Discovery of a covalent inhibitor of KRASG12C (AMG 510) for the treatment of solid tumors, J. Med. Chem. 63 (2020) 52–65, https://doi.org/10.1021/acs.jmedchem.9b01180.

[6] D.S. Hong, M.G. Fakih, J.H. Strickler, J. Desai, G.A. Durm, G.I. Shapiro, G. S. Falchook, T.J. Price, A. Sacher, C.S. Denlinger, Y.-J. Bang, G.K. Dy, J.C. Krauss, Y. Kuboki, J.C. Kuo, A.L. Coveler, K. Park, T.W. Kim, F. Barlesi, P.N. Munster, S. S. Ramalingam, T.F. Burns, F. Meric-Bernstam, H. Henary, J. Ngang, G. Ngarmchamnanrith, J. Kim, B.E. Houk, J. Canon, J.R. Lipford, G. Friberg, P. Lito, R. Govindan, B.T. Li, KRAS G12C inhibition with sotorasib in advanced solid tumors, N. Engl. J. Med. (2020), https://doi.org/10.1056/nejmoa1917239.

[7] C.J. Lucas, J.H. Martin, Pharmacokinetic-guided dosing of new oral cancer agents, J. Clin. Pharmacol. 57 (2017) S78–S98, https://doi.org/10.1002/jcph.937.

[8] T. Hendrayana, A. Wilmer, V. Kurth, I.G.H. Schmidt-Wolf, U. Jaehde, Anticancer dose adjustment for patients with renal and hepatic dysfunction: From scientific evidence to clinical application, Sci. Pharm. 85 (2017), https://doi.org/10.3390/scipharm85010008.

[9] E. Masson, W.C. Zamboniz, Pharmacokinetic optimisation of cancer chemotherapy effect on outcomes, Clin. Phormacokinet. 32 (1997) 324–343, https://doi.org/10.2165/00003088-199732040-00005.

[10] N. Madhyastha, S. Kumar Samantha, S. Dittakavi, M. Markose, S. Rangnathrao Mallurwar, M. Zainuddin, R. Mullangi, Validated HPLC-MS/MS method for quantitation of AMG 510, a KRAS G12C inhibitor, in mouse plasma and its application to a pharmacokinetic study in mice, Biomed. Chromatogr. (2020), e5043, https://doi.org/10.1002/bmc.5043.

[11] I.A. Retmana, J.H. Beijnen, R.W. Sparidans, Chromatographic bioanalytical assays for targeted covalent kinase inhibitors and their metabolites, J. Chromatogr. B. 1162 (2020), 122466, https://doi.org/10.1016/j.jchromb.2020.122466.

[12] W. Li, N. Perpinioti, A.H. Schinkel, J.H. Beijnen, R.W. Sparidans, Bioanalytical assay for the new-generation ROS1/TRK/ALK inhibitor repotrectinib in mouse plasma and tissue homogenate using liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1144 (2020) 122098. https://doi.org/10.1016/j.jchromb.2020.122098.

[13] R. Şentürk, Y. Wang, A.H. Schinkel, J.H. Beijnen, R.W. Sparidans, Quantitative bioanalytical assay for the selective RET inhibitors selpercatinib and pralsetinib in mouse plasma and tissue homogenates using liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1147 (2020) 122131. https://doi.org/10.1016/j.jchromb.2020.122131.

[14] FDA, CDER, Bioanalytical method validation guidance for industry, (2018). https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf (accessed May 4, 2020).

[15] European Medicine Agency, Guideline on bioanalytical method validation, (2011). http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf (accessed May 10, 2020).

[16] A. Tan, J.C. Fanaras, Use of high-pH (basic/alkaline) mobile phases for LC–MS or LC–MS/MS bioanalysis, Biomed. Chromatogr. 33 (2019), e4409, https://doi.org/10.1002/bmc.4409.

[17] B. Dogan-Topal, W. Li, A.H. Schinkel, J.H. Beijnen, R.W. Sparidans, Quantification of FGFR4 inhibitor BLU-554 in mouse plasma and tissue homogenates using liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1110–1111 (2019) 116–123. https://doi.org/10.1016/j.jchromb.2019.02.017.