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Simultaneous quantification of dabrafenib and trametinib in human plasma using high-performance liquid chromatography–tandem mass spectrometry

C.M. Nijenhuis[a,*], H. Haverkate[a], H. Rosing[a], J.H.M. Schellens[b,c], J.H. Beijnen[a,c]

[a] Department of Pharmacy & Pharmacology, Antoni van Leeuwenhoek/The Netherlands Cancer Institute and MC Slotervaart, Amsterdam, The Netherlands
[b] Division of Clinical Pharmacology, Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
[c] Division of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Department of Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

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ABSTRACT

Dabrafenib (Tafinlar®) and trametinib (Mekinist®) are registered for the treatment of patients with BRAF V600 mutation positive unresectable or metastatic melanoma. To support therapeutic drug monitoring (TDM) and clinical pharmacological trials, an assay to simultaneously quantify dabrafenib and trametinib in human plasma using liquid chromatography tandem mass spectrometry was developed and validated.

Human plasma samples were collected on an outpatient base and stored at nominally −20 °C.

Analytes and internal standards (stable isotope labeled compounds) were extracted with TBME. After snap freezing the samples in a dry ice-ethanol bath, the organic layer was transferred to a clean tube and evaporated under a gentle stream of nitrogen gas. The dry extract was then reconstituted with 100 μL acetonitrile and 5 μL of the final extract was injected and separated on a C18 column with gradient elution, and analyzed with triple quadrupole mass spectrometry in positive-ion mode.

The validated assay ranges from 50 to 5000 ng/mL for dabrafenib and 0.5–50 ng/mL for trametinib were linear, and correlation coefficient (r^2) of 0.996 or better. At all concentrations of both analytes the biases were within ±15% of the nominal concentrations and precisions were ≤15%. All results were within the acceptance criteria of the latest US FDA guidance and EMA guidelines on method validation. Dabrafenib was found to degrade under the influence of light in different organic solvents and at least seven degradation products were detected.

In conclusion, the described method to simultaneously quantify dabrafenib and trametinib in human plasma was successfully validated and applied for therapeutic drug monitoring in cancer patients treated with dabrafenib and trametinib.

1. Introduction

Dabrafenib (Tafinlar®) is an inhibitor of mutated serine/threonine-protein kinase B-Raf (BRAF) and trametinib (Mekinist®) is an inhibitor of MAPK kinase (MEK). Both drugs are registered for the treatment of patients with BRAF V600 mutation positive unresectable or metastatic melanoma. The use of BRAF inhibitors, such as vemurafenib and dabrafenib, has been associated with prolonged survival and progression-free survival, respectively, in phase 3 trials [1–3]. Trametinib resulted in improved progression-free survival and overall survival [4]. In spite of these advances, 50% of patients who are treated with BRAF inhibitors or MEK inhibitors develop resistance leading to disease progression within 6–7 months after initiation of the treatment. Since trametinib mediates blockade of MEK, which is downstream of BRAF in the MAPK pathway, the combination of trametinib and dabrafenib was investigated. Combination therapy of dabrafenib and trametinib was associated with a longer median progression-free survival than dabrafenib monotherapy [5].

To further investigate the pharmacokinetics of both drugs and to support therapeutic drug monitoring (TDM) an assay for the simultaneous quantification of dabrafenib and trametinib is needed. Two

* Corresponding author at: Antoni van Leeuwenhoek/The Netherlands Cancer Institute, Department of Pharmacy & Pharmacology, Louwesweg 6, 1066 EC Amsterdam, The Netherlands.
E-mail address: Cynthia.Nijenhuis@slz.nl (C.M. Nijenhuis).

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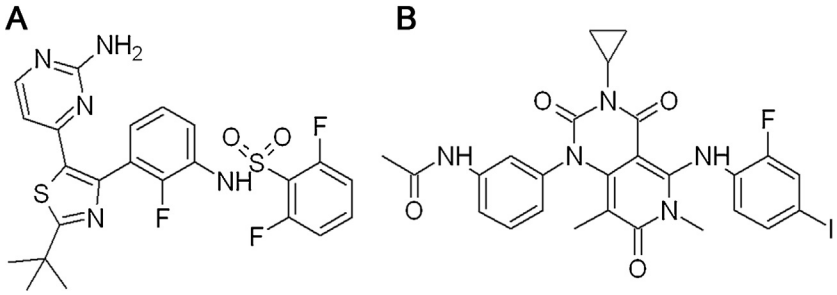


Fig. 1. Chemical structures of dabrafenib and trametinib.

bioanalytical assays for dabrafenib were reported before by Mittapalli et al. [6] but without bioanalytical details and by Sparidans et al. [7]. No bioanalytical assays have been described for trametinib as far as we know.

Herein we describe the first bioanalytical assay for the quantification of dabrafenib and trametinib that was fully validated according to the latest FDA and EMA guidelines on bioanalytical method validation [8,9].

2. Materials and methods

2.1. Chemicals

Dabrafenib and trametinib (Fig. 1) were obtained from Sequoia Research Products (Pangbourne, UK) and $^{2}H_9$-dabrafenib and $^{13}C_6$-trametinib, used as internal standards (IS) for this assay, were manufactured by AlsaChim (Illkirch, France). Methanol and water (HPLC grade), used to prepare mobile phases, were obtained from Biosolve Ltd (Valkenswaard, The Netherlands). Dimethylsulfoxide (DMSO) for stocks and working solutions, ammonium acetate (>98%) used to prepare mobile phase A, and tert-butyl methyl ether (TBME), used for sample preparation, were purchased from Merck (Darmstadt, Germany). Water (distilled) used for sample preparation originated from B. Braun Medical (Melsungen, Germany). K_2EDTA plasma was obtained from the Medical Center Slotervaart (Amsterdam, The Netherlands).

2.2. Stock solutions, calibration standards and quality control samples

All stock and working solutions should be made and stored in amber colored material. Separate stock solutions for calibration standards and quality control samples (QC samples) of 1 mg/mL for dabrafenib and 0.5 mg/mL for trametinib, were prepared in DMSO. These stock solutions were further diluted with DMSO to obtain working solutions.

Stock solutions of the internal standards $^{2}H_9$-dabrafenib and $^{13}C_6$-trametinib were prepared at a concentration of 2 mg/mL in DMSO. The IS working solution contained 4000 ng/mL $^{2}H_9$-dabrafenib and 40 ng/mL $^{13}C_6$-trametinib in DMSO.

Internal standard stock solutions and working solutions for all analytes were stored at −20 °C.

Calibration standards were spiked directly after the preparation of the working solutions. A volume of 100 μL of calibration standard working solution was added to 1900 μL control human K_2EDTA plasma to obtain concentrations of 50.0, 100, 250, 500, 1000, 2500, 4000 and 5000 ng/mL for dabrafenib and 0.500, 1.00, 2.50, 5.00, 10.0, 25.0, 40.0 and 50.0 ng/mL for trametinib. For sample pre-treatment 100 μL aliquots were used.

The QC samples were prepared by adding QC working solution (100 μL) to control human plasma (1900 μL). Final concentrations at the lower limit of quantification (LLOQ), QC low, QC mid, QC high and QC above the upper limit of quantification (>ULOQ) were 50.0, 150, 750, 3750 and 25000 ng/mL for dabrafenib and 0.500, 1.50, 7.50, 37.5 and 250 ng/mL for trametinib. The QC samples were stored in aliquots of 100 μL at nominally −20 °C.

2.3. Sample preparation

Immediately after sample collection in the clinic by venipuncture, the plasma samples were centrifuged for 10 min at 1700*g* at 4 °C. After centrifugation, the plasma was isolated and stored in amber colored containers at −20 °C pending analysis.

Table 1
Mass spectrometric parameters for the analysis of dabrafenib and trametinib with the internal standards $^{2}H_9$-dabrafenib and $^{13}C_6$-trametinib.

General settings	API4000			
Run duration	6.4 min			
Ionspray voltage	+5.0 kV			
Nebulizer gas	60 psi			
Turbo gas	40 psi			
Curtain gas	40 psi			
Collision gas	10 psi			
Temperature	500 °C			
Analyte specific parameters	**Dabrafenib**	**$^{2}H_9$-Dabrafenib**	**Trametinib**	**$^{13}C_6$-Trametinib**
Parent mass	520.2 *m/z*	529.2 *m/z*	616.2 *m/z*	622.2 *m/z*
Product mass	307.3 *m/z*	316.3 *m/z*	491.2 *m/z*	497.3 *m/z*
Dwell time	100 ms	100 ms	100 ms	100 ms
Collision energy	30 V	30 V	45 V	45 V
Collision exit potential	40 V	40 V	30 V	30 V
Declustering potential	20 V	20 V	91 V	91 V
Typical retention time	3.50 min	3.50 min	4.00 min	4.00 min

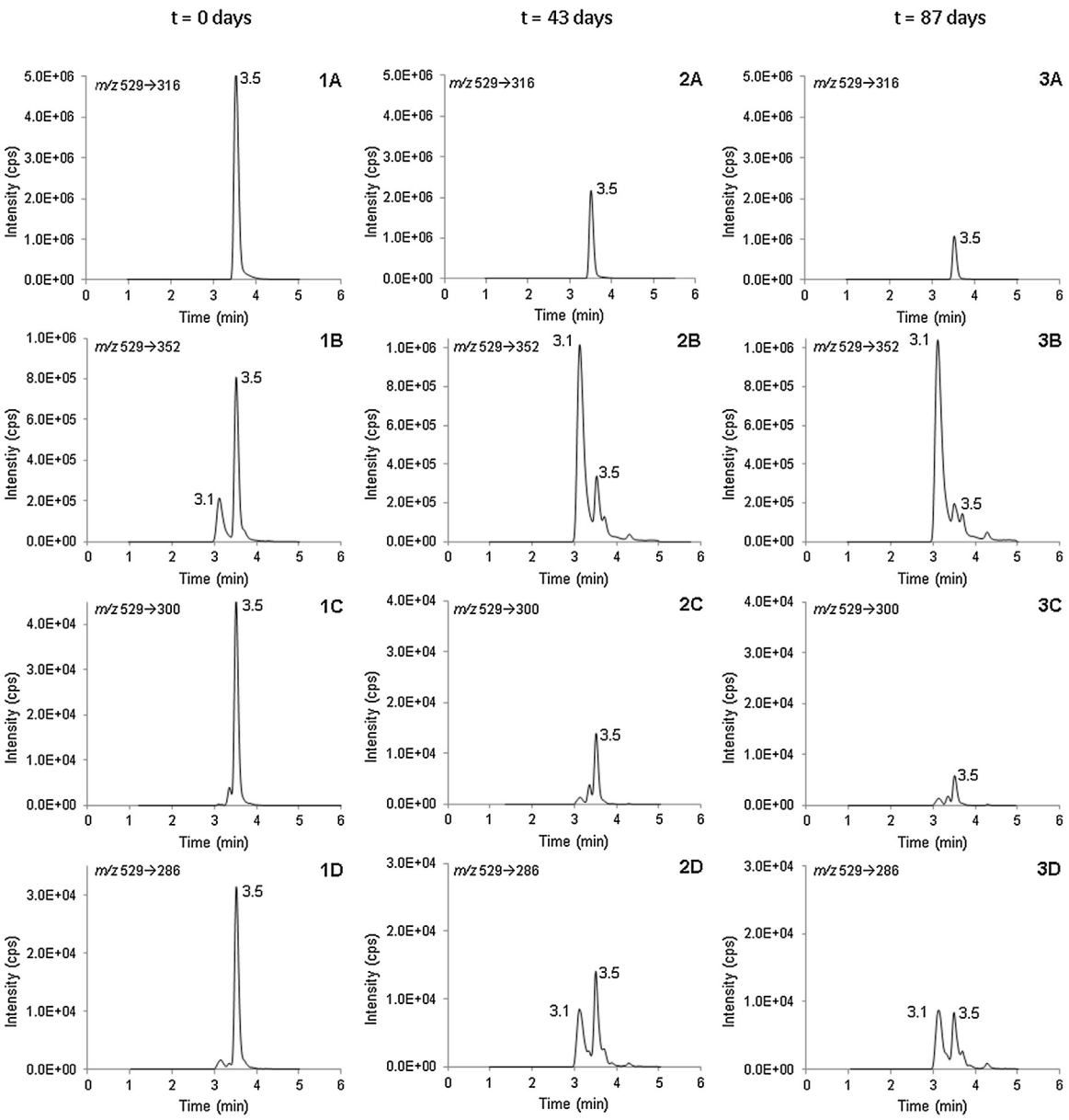


Fig. 2. Chromatograms of $^{2}H_{9}$-dabrafenib in methanol at different time points. $^{2}H_{9}$-Dabrafenib was freshly diluted (1) in methanol at a concentration of 4000 ng/mL and stored at −20 °C for 43 days (2) and 87 days (3). These three solutions were analyzed and monitored at different transitions; *m/z* 529 → 316 (A), *m/z* 529 → 352 (B), *m/z* 529 → 300 (C) and *m/z* 529 → 286 (D). The validated mass transition (A1) shows one peak but this peak decreases over time (2A and 3A). Transition *m/z* 529 → 352 (1B) shows a peak at 3.1 min and 3.5 min, but the peak ratio changes over time and finally the peak at 3.1 min is larger than the peak at 3.5 min (2B and 3B). Transition *m/z* 529 → 300 (1C) shows one peak which decreases over time (2C and 3C). Transition *m/z* 529 → 286 (D) initially shows one peak, but over time another peak appears at 3.1 min (2D) and the peak ratio changes over time (3D).

Prior to processing the samples was thawed on an ice-water bath and a 100 μL aliquot was transferred to an amber colored container of 2 mL. A volume of 20 μL of IS working solution (4000 ng/mL $^{2}H_{9}$-dabrafenib and 40 ng/mL $^{13}C_{6}$-trametinib) was added to the plasma sample. To extract the analytes and internal standards from the biomatrix, a volume of 1 mL of TBME was added. Then the samples were vortex-mixed, shaken (10 min at 1250 rpm) and centrifuged (5 min at 20 °C and 23,100*g*). After snap freezing the samples in an dry ice-ethanol bath, the organic layer was removed and evaporated under a gentle stream of nitrogen gas (approximately 15 min at 40 °C). The dry extract was then reconstituted with 100 μL acetonitrile and 5 μL of the final extract was injected. For the sample preparation and sample storage amber colored material should be used.

2.4. Liquid chromatography–tandem mass spectrometry

The chromatographic separation was carried out using a HP1100 binary pump, a degasser, a HP1100 autosampler and a switching valve (Agilent technologies, Palo Alto, CA, USA). The autosampler temperature was kept at 4 °C and the column oven at 40 °C. The mobile phase A consisted of 10 mM ammonium acetate in water and mobile phase B was methanol. Gradient elution was applied at a flow rate of 0.25 mL/min through a Gemini C18 column (110 Å, 50 × 2.0 mm ID, particle size 5.0 μm; Phenomenex, Torrance, CA, USA) with an additional Gemini C18 guard column (4 × 2.0 mm ID). The following gradient was applied: 50% to 80% B, 0.25 mL/min (0–0.5 min); 80% B, 0.25 mL/min (0.5–2.5); 80–95% B, 0.25 mL/min (2.5–2.6 min), 95% B, 0.25 mL/min (2.6–4.6 min); 95% B, 0.25–0.5 mL/min (4.6–4.7 min); 95% B,

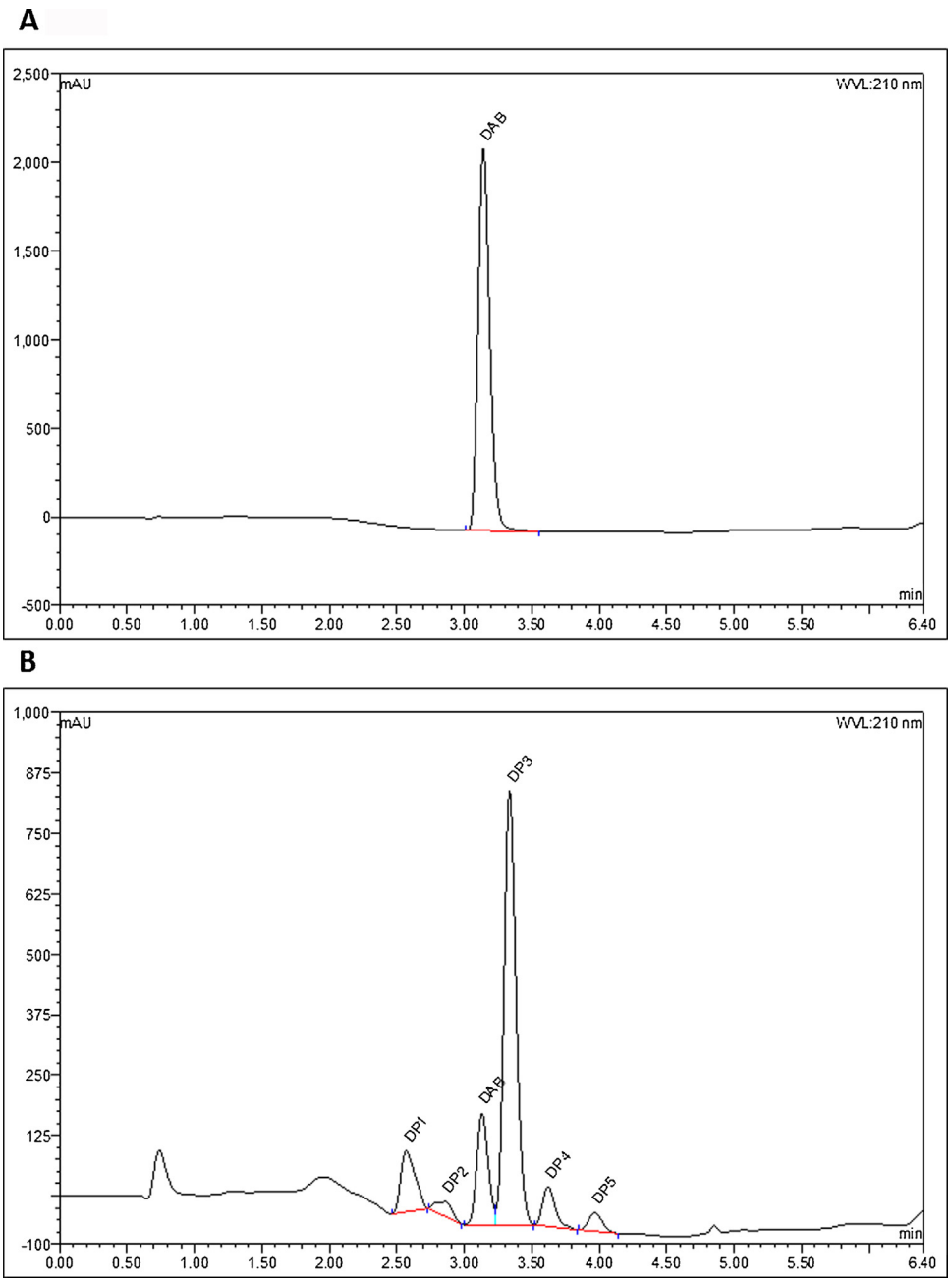


Fig. 3. UV chromatogram of 1 mg/mL dabrafenib in methanol at (Fig. 3A) t = 0 and (Fig. 3B) t = 144 h exposed to light. Dabrafenib (DAB) is eluting after 3.1 min and at least 5 degradation products (DP) were detected after respectively 2.6 min, 2.9 min, 3.3 min, 3.6 min and 4.0 min. DP2 is proposed to be hydroxyl-dabrafenib and DP3 is dabrafenib with a loss of [HF]. The structure of DP1, DP4 and DP5 could not be elucidated.

0.5 mL/min (4.7–5.2 min); 95–50% B, 0.5 mL/min (5.2–5.3 min); 50% B, 0.5 mL/min (5.3–6.3 min); 50% B, 0.5–0.25 mL/min (6.3–6.4 min). The divert valve directed the flow to the mass spectrometer between 1.0 and 5.0 min and the remainder to the waste container.

Dabrafenib and trametinib were analyzed on an API4000 quadrupole mass spectrometer (MS) (Sciex, Thornhill, ON, Canada). This instrument is equipped with a turbo ion spray (TIS) interface, operating in positive mode and configured in multiple reaction monitoring (MRM). The LC–MS/MS data were acquired and processed with Analyst^{TM} software (Sciex). Table 1 summarises the MS operating parameters.

2.5. Validation procedures

The assay was fully validated for calibration model, accuracy and precision, lower limit of quantitation, specificity and selectivity, dilution integrity, carry-over, matrix effect and stability. This was in accordance to the FDA and latest EMA guidelines on bioanalytical method validation [8,9].

The matrix factor was determined by using the following equation:

$$\mathrm{MF} = \frac{\text{Area of a processed blank sample spiked with neat solution (matrix present)}}{\text{Area of neat solution (matrix absent)}} \quad (1)$$

Table 2
MS and UV data, and proposed photodegradation products of dabrafenib. The peak number corresponds to the peak numbers displayed in Fig. 3.

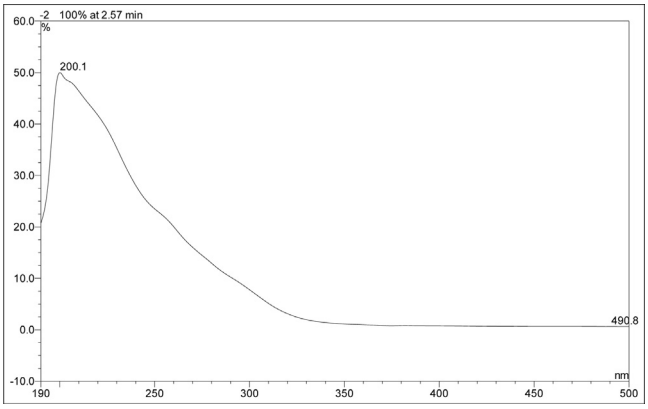
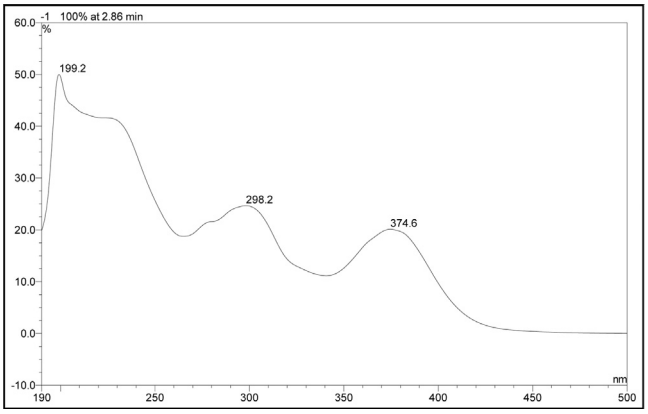
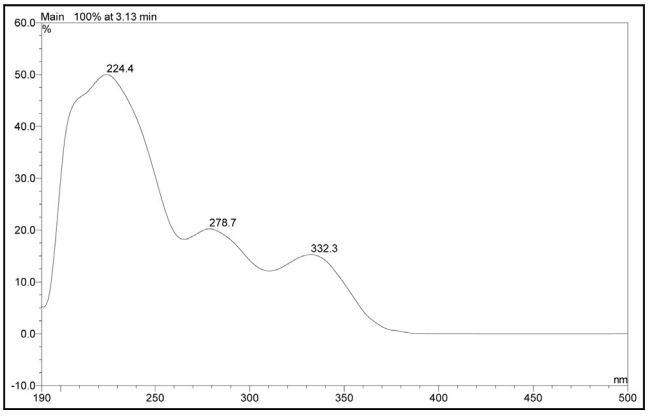
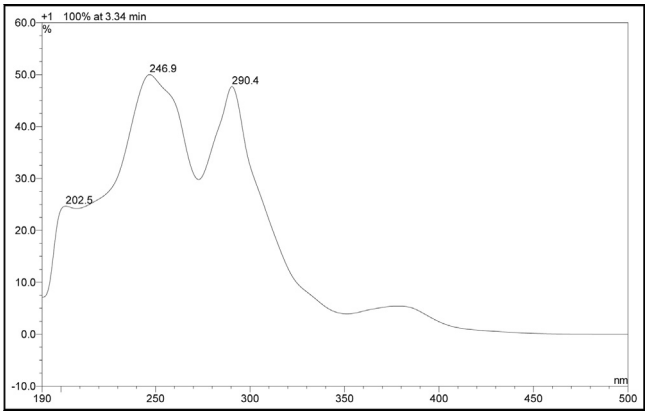
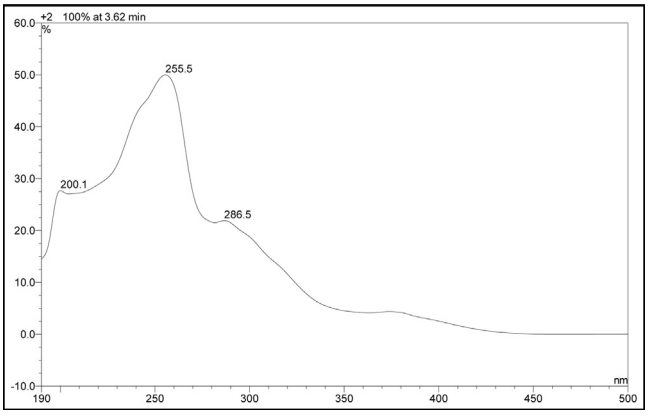
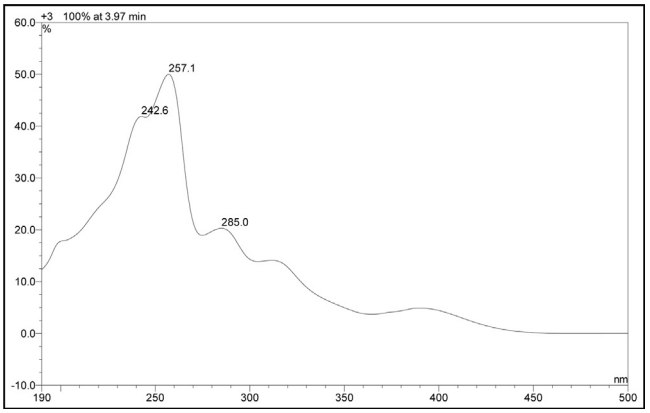
DP no	(Proposed) identity	RT (min)	Observed *m*/*z*	UV spectrum
1	Unknown	2.6	MS^1: 598 MS^2: 556	
2	Hydroxy-dabrafenib	2.9	MS^1: 536 MS^2: 359	
–	Dabrafenib	3.1	MS^1: 520 MS^2: 343, 307	
3	Dabrafenib -[HF]	3.3	MS^1: 500 MS^2: 323	

Table 2 (*Continued*)

DP no	(Proposed) identity	RT (min)	Observed *m*/*z*	UV spectrum
4	Unknown	3.6	UK	
5	Unknown	3.4	UK	

Abbreviations: DP: degradation product, no: number, RT: retention time, UK: unknown.

2.6. Photodegradation of dabrafenib

To investigate the stability of dabrafenib, 1 mg/mL solutions for dabrafenib were prepared in DMSO, methanol, water-methanol (50:50, v/v), ethanol and water-ethanol (50:50, v/v). These stock solutions were divided over two autosampler vials: one amber colored vial and one transparent vial. The vials were stored at ambient temperatures, in daylight at a window bench, and analyzed up to 144 h after preparation using a chromatography-diode array detector (LC-DAD) method for which the same chromatographic conditions were used as described earlier. The wavelengths were monitored in a range from 100 to 800 nm. After 144 h of storage, the solutions were analyzed using an ion trap mass spectrometer detector (LTQ XL, Thermo Electron, Waltham, MA, USA) to assess the parent masses of dabrafenib and its degradation products.

2.7. Clinical application

The purpose of this assay is to support TDM and clinical pharmacokinetic studies of dabrafenib and trametinib in patients with melanoma. As part of routine clinical care, K_2EDTA blood samples (4 mL) were collected for pharmacokinetic monitoring from patients who were treated with dabrafenib and trametinib at the Antoni van Leeuwenhoek–Netherlands Cancer Institute. This was approved by the medical ethical committee of this institution. Plasma samples were collected and immediately stored at −20 °C pending analysis. Before analysis samples were thawed on an ice-water bath and a 100 μL aliquot was taken and processed as described in this report.

3. Results and discussion

3.1. Development

3.1.1. Mass spectrometry

Simultaneous quantification of dabrafenib and trametinib was complicated due to the large differences (50-fold) in plasma concentrations and because dabrafenib is a better MS responder than trametinib. Mass spectrometric parameters were optimized for each analyte by performing direct infusion (methanol-0.1% formic acid in water (70:30, v/v)) and flow injection analysis (methanolic solution). General settings were chosen in favour of trametinib, since for this compound the lowest target concentrations had to be reached. The molecular ions ($[M+H]^+$) observed for dabrafenib and trametinib at *m*/*z* 520 and 616, respectively, were used to generate product ion spectra. In order to achieve high specificity and sensitivity, the MRM scan mode was applied to monitor for each analyte the mass transition to the product ion with the highest abundance in the product ion scan. For dabrafenib the product ion at *m*/*z* 307 were optimized and for trametinib *m*/*z* 491. However, with these optimal settings the calibration model of dabrafenib was non-linear due to saturation of the detector. To prevent saturation of the dabrafenib response, we lowered the collision energy (CE) from 47 to 30 V. The collision exit potential (CXP) was increased from 20 to 40 V and the declustering potential (DP) lowered from 116 to 20 V. These apparent non-optimal settings resulted in accurate and precise quantification of dabrafenib, despite the differences in ionization and target concentrations ranges between dabrafenib and trametinib.

Table 3
Assay performance data for the analysis of dabrafenib and trametinib in human plasma.

			Intra-assay		Inter-assay	
Analyte	Nom. conc. (ng/mL)	n	Bias[a] (%)	CV[a] (%)	Bias(%)	CV(%)
Dabrafenib	52.4	15	−8.6 to −5.9	1.7–2.5	−7.2	2.4
	157	15	6.5 to 10.6	0.7–2.1	8.7	2.1
	786	15	5.5 to 8.1	1.2–1.6	6.6	1.6
	3930	15	−3.3 to −0.9	0.6–1.7	−2.4	1.5
Trametinib	0.503	15	−6.2 to −1.9	3.4–6.9	−4.3	5.6
	1.51	15	−4.4 to −2.3	2.4–4.8	−3.1	3.5
	7.55	15	−3.2 to −2.3	1.5–3.4	−2.9	2.3
	37.8	15	−4.1 to −2.7	1.4–1.9	−3.3	1.7

Nom.: nominal; Conc.: concentration; n: number of replicates.
[a] If multiple validation runs were performed, the range of accuracies and precisions was listed.

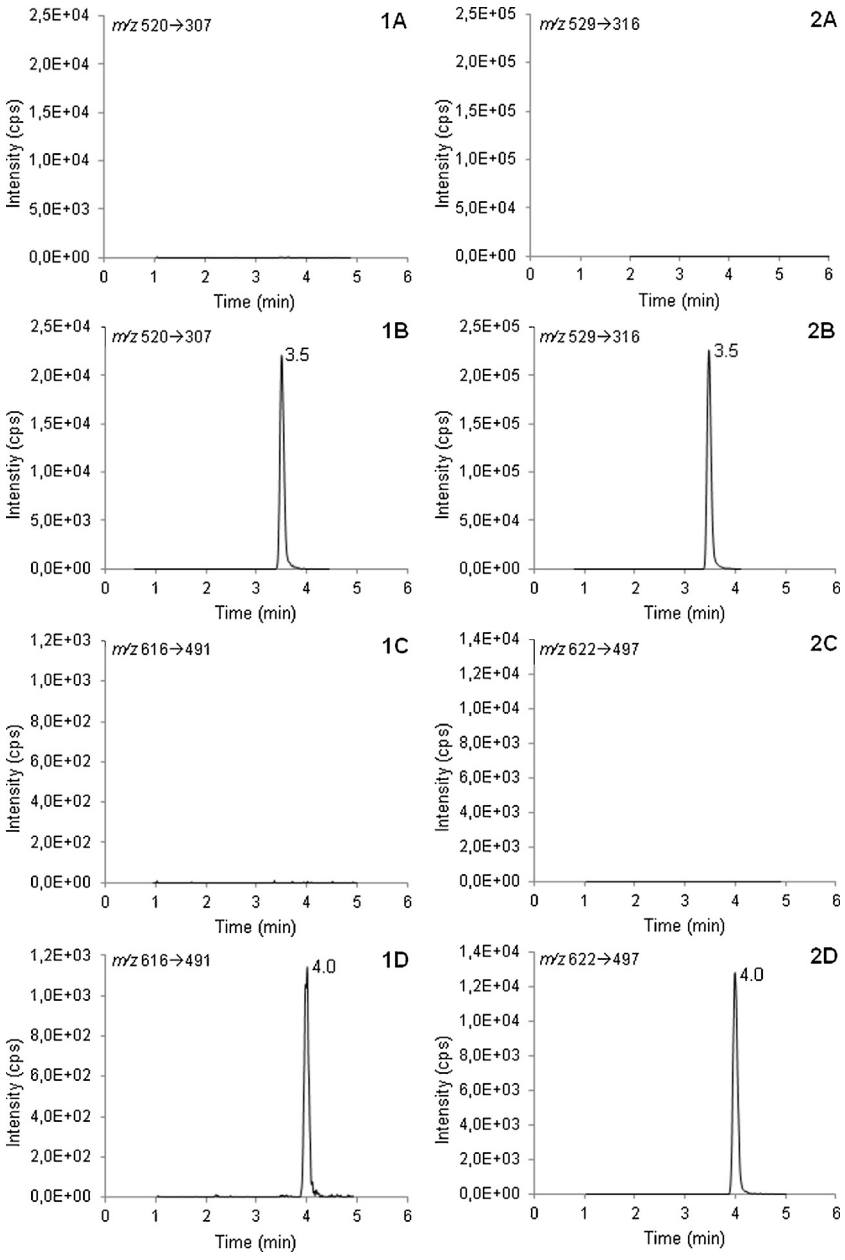


Fig. 4. Chromatograms from plasma samples in blank samples of dabrafenib (A1) and the internal standard (2A) and at the LLOQ of dabrafenib (1B, 50 ng/mL) and of the internal standard (2B, 4000 ng/mL). In addition chromatrograms are shown from plasma samples in blank samples of trametinib (1C) and the internal standard (2C) and at the LLOQ of trametinib (1D, 0.5 ng/mL) and of the internal standard (2D, 40 ng/mL).

Table 4
Stability data for dabrafenib and trametinib in plasma. All experiments in plasma were performed in triplicate at low and high concentration levels.

Conditions	Matrix	Analyte	Nominal conc. (ng/mL)	Measured conc. (ng/mL)	Bias(%)	CV(%)	n
Stock solutions							
−20 °C, 3 m	DMSO	Dabrafenib	0.495E + 6	0.519E + 6	4.8	2.8	3
		Trametinib	1.02E + 6	1.04E + 6	2.3	2.0	3
Plasma							
3 freeze–thaw cycles (−20 °C/RT)	Plasma	Trametinib	1.51	1.44	−4.4	1.7	3
			37.8	36.1	−4.1	1.8	3
		Dabrafenib	157	148	−5.5	2.4	3
			3930	3710	−5.6	1.9	3
2–8 °C, 6 h	Plasma	Dabrafenib	157	156	−0.8	3.0	3
			3930	3823	−2.7	0.8	3
RT, 6 h	Plasma	Dabrafenib	157	141	−10.3	0.7	3
			3930	3463	−11.9	1.1	3
RT, 24 h	Plasma	Trametinib	1.51	1.38	−8.6	0.7	3
			37.8	36.0	−4.8	2.6	3
		Dabrafenib	157	126	**−20.0**	2.6	3
			3930	3367	−14.3	0.3	3
−20 °C, 20 d	Plasma	Trametinib	1.51	1.43	−5.5	1.1	3
			37.8	35	−7.2	0.3	3
		Dabrafenib	157	161	2.3	2.0	3
			3930	3773	−4.0	0.6	3
2–8 °C, 2 d	Final extract	Trametinib	1.51	1.31	−13.2	1.5	3
			37.8	33.0	−12.3	1.7	3
2–8 °C, 6 d	Final extract	Trametinib	1.51	1.22	**−19.2**	0.8	3
			37.8	32.0	**−16.0**	1.3	3
		Dabrafenib	157	163	**3.8**	1.2	3
			3930	3810	**−3.1**	3.9	3
2–8 °C, 8 d	Dry extract	Trametinib	1.51	1.37	−9.3	1.9	3
			37.8	34.0	−10.8	2.4	3
		Dabrafenib	157	172	9.8	0.9	3
			3930	3830	−2.5	1.1	3

Conc.: concentration; C.V.: Coefficient of Variation; n: number of replicates; m: months; **Bold**: not accepted.

3.1.2. HPLC

We used a Gemini C18 column during development and tested 10 mM ammonium acetate and 10 mM ammonium hydroxide as aqueous phases of eluent. The response for dabrafenib and trametinib increased 5.0–5.5-fold when using an alkaline mobile phase. However, the peaks also showed tailing which could not be prevented by changing the gradient. Therefore 10 mM ammonium acetate in water in combination with methanol was selected. Signal to noise ratios measured in the trametinib LLOQ samples were acceptable (>10) because low noise levels were obtained.

3.1.3. Photodegradation of dabrafenib

During the development of the analytical method transparent material was used and working solutions was initially made in methanol. However stability in dried extract and final extract showed deviations for dabrafenib of approximately −30% and −50% respectively (after 24 h at 2–8 °C). It should be noted though that the results for trametinib were all within the acceptance criteria. To investigate the instability of dabrafenib, working solutions in methanol containing dabrafenib and its internal standard was analyzed after 42 and 83 days of storage at −20 °C. Different transitions were monitored and results of the internal standard of dabrafenib are shown in Fig. 2. Several dabrafenib related degradation products were found in these windows indicating that the compound is unstable in methanol when kept at −20 °C.

The stability of dabrafenib was further investigated at a concentration of 1 mg/mL at ambient temperature in different organic solutions; DMSO, methanol, water-methanol (50:50, v/v), ethanol and water-ethanol (50:50, v/v), exposed and protected from light.

After 144 h, no degradation was observed in all tested organic solutions when the vials were protected from light. However, exposed to light, profound degradation of dabrafenib was measured in all alcohol containing solutions. Fig. 3 shows a UV chromatogram at 220 nm of a 1 mg/mL dabrafenib in methanol directly after spiking and after 144 h of light exposure. At least 5 degradation products are formed and in Table 2 parent masses and UV spectra from the main degradation products are presented. Similar degradation patterns were observed in alcohol containing solutions whereas in DMSO dabrafenib is fairly stable.

The MS spectrum of degradation product 2 (R_t = 2.9 min) showed an addition of 16 Da to the mass of dabrafenib indicating addition of a hydroxyl group. This theory is corroborated by the UV spectrum of this peak which shows absorption peaks at longer wavelengths which might point to hydroxyl addition and that this product is only formed in methanol and ethanol. We also propose the formation of a degradation product with the loss of fluorine. The MS spectrum of degradation product 3 (R_t = 3.3 min) showed a loss of 20 Da compared to dabrafenib, indicating the loss of HF. The MS data also showed a degradation product co-eluting with dabrafenib with a loss of 2 Da, indicating the loss of 2 $[H]^+$. The structure of the other degradation projects could not be elucidated using these methods.

Based on these investigations, it was decided to prepare the stock and working solutions of dabrafenib and its internal standard in DMSO. Using DMSO as solvent, the stability problems were not solved completely, because in time, when stored at −20 °C, dabrafenib tends to adsorb to the container (PP tubes) at low concentrations. Therefore, calibration standards should be prepared immediately after the preparation of the working solutions. In

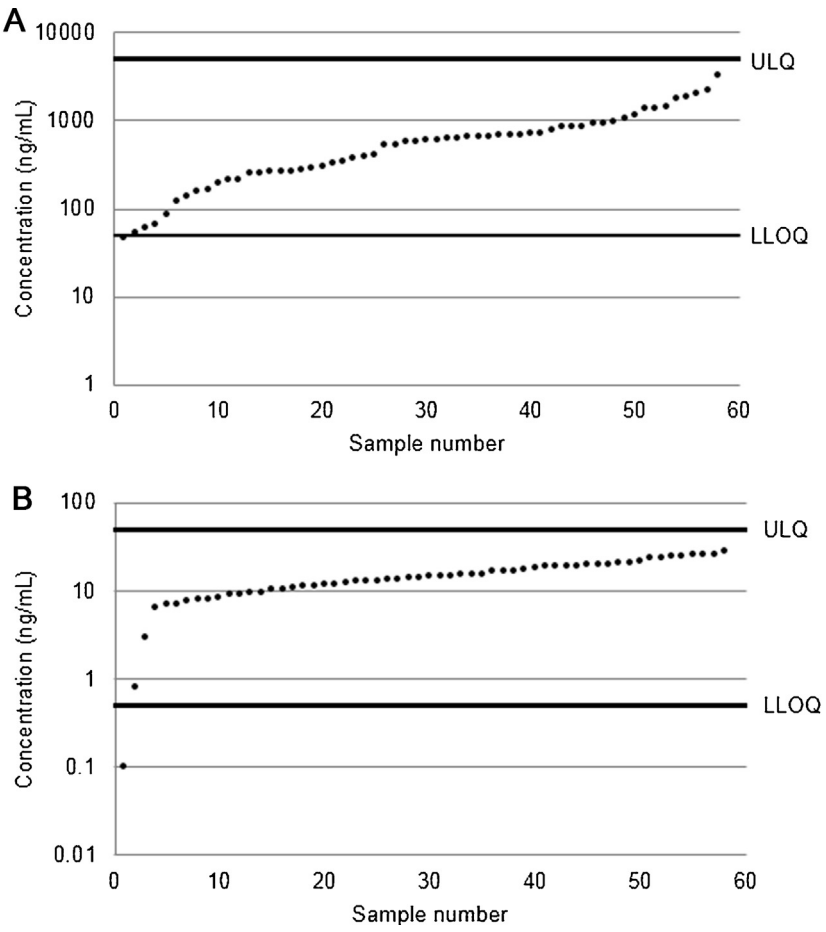


Fig. 5. Plasma concentrations of dabrafenib (A) and trametinib (B) of individual samples. The solid lines show the LLOQ and ULQ of both analytes.

addition 100% acetonitrile was chosen as reconstitution solvent, to minimize degradation in the final extract after sample preparation.

3.2. Validation procedures

3.2.1. Calibration model

Calibration standards were prepared and analyzed in duplicate in three analytical runs. The linear regression of peak area ratios versus the concentration (x) was weighted ($1/x^2$) to obtain the lowest total bias with the simplest model. The calibration range of dabrafenib in plasma was 50–5000 ng/mL and of trametinib 0.5–50 ng/mL. Calibration curves were accepted if 75% of the non-zero calibration standards, including a LLOQ and an ULOQ, had a bias within ±15% of the nominal concentration (±20% at the LLOQ) [8,9]. All calibration curves (n = 3) of the analytes met these criteria and correlation coefficients (r^2) of 0.996 and 0.998, for dabrafenib and trametinib respectively, or better were obtained.

3.2.2. Accuracy and precision

To assess the accuracy and precision of the assays, five replicates of QC LLOQ, QC low, QC mid and QC high in plasma were analyzed in three analytical runs.

Table 3 summarises the intra- and inter-assay accuracies and precisions of the assay. The biases and CVs were within the acceptance criteria (within ±20% and ≤20%, respectively, at the LLOQ level and within ±15% and ≤15% at the other QC levels).

3.2.3. Lower limit of quantitation

The analytes responses at the LLOQ were at least 5 times the response compared to a blank response in three validation runs. The lowest signal to noise ratio for dabrafenib was 25 and for trametinib 90. Fig. 4 shows representative ion chromatograms of dabrafenib and trametinib in QC LLOQ samples and double blank samples.

3.2.4. Specificity and selectivity

Six different batches of control plasma (pooled) were spiked at the LLOQ level with dabrafenib and trametinib to investigate the specificity and selectivity. Single determinations were performed. The mean deviations from the nominal concentrations from the plasma assay were 14.9% and 14.9% with CV values of 6.5% and 5.3%, respectively. There were no peaks observed with areas >20% of the LLOQ in double-blank samples of these batches in any of the analytes and also no interferences were detected at the retention times of the internal standards for both assays. Selectivity was therefore considered acceptable.

Cross-analyte and internal standard interference was tested by spiking control human plasma at ULOQ level with all dabrafenib and trametinib and all IS separately at the nominal concentration (one level). The cross analyte and internal standard interference at the retention time of both analytes were less than 20% of the peak area of the LLOQ level. For the internal standard the interference was less than 5%. For dabrafenib and trametinib the cross analyte and internal standard interference was considered acceptable.

3.2.5. Dilution integrity

Five replicate plasma samples of dabrafenib and trametinib at QC >ULOQ level were diluted 10-fold with control human plasma (10 μL of sample was added to 90 μL control human matrix). For the plasma assay 100 μL was processed. For dabrafenib the intra-assay bias and CVs were −9.0% and 9.1% respectively and for trametinib

−12.3% and 9.1% respectively. The bias and CVs were within ±15% and ≤15% which indicates that the study samples can be diluted 10 times in control matrix with acceptable accuracy and precision.

3.2.6. Carry over

Carry over was investigated by injecting two double blank samples after a ULOQ sample in three analytical runs. Eluting peaks with areas >20% of the LLOQ were not observed in the blank samples injected after ULOQ samples. Thus, the criteria for carryover were fulfilled.

3.2.7. Matrix factor

The matrix factor (MF) was determined in six plasma batches (pooled), at low and high concentration levels of dabrafenib and trametinib. Single determinations were performed. Processed blank samples were spiked with working solutions and compared to matrix free neat solutions.

The maximal CV of the internal standard-normalised MF calculated from the six plasma batches for the low and high concentration was respectively 3.0% and 1.9% and fulfilled the criteria (≤15%). The internal standard-normalized MF ranged for both analytes from 0.961 to 1.09. These results (MF around 1) indicate that the stable isotopes as internal standards are most effective in minimizing the influence of matrix effects.

3.2.8. Stability

The results of the stability experiments are displayed in Table 4. Since non-freshly prepared calibration standards were used for the validation the stability of dabrafenib and trametinib at −20 °C in plasma during the time of the validation had to be confirmed (bias and CV within ±15% and ≤15%). Dabrafenib and trametinib were stable at −20 °C in plasma for at least 20 days. Trametinib was stable in plasma for at least 24 h at ambient temperature, but dabrafenib was not. Therefore stability for 6 h at ambient temperature and at 2–8 °C (refrigerator) was tested. Dabrafenib was stable for at least 6 h at ambient temperature and at 2–8 °C. However after 6 h at ambient temperature the dabrafenib concentration decreased with 10–11%. Therefore during sample collection the whole blood samples should be centrifuged at 4 °C to collect plasma. In addition samples should be thawed at 2–8 °C and prepared on an ice-bath and during all preparation steps amber tubes should be used. Under these conditions the analytes are stable. Dabrafenib and trametinib were stable for at least eight days in dry extract at 2–8 °C. Dabrafenib was stable for at least six days in final extract at 2–8 °C and trametinib only 2 days at 2–8 °C. Long term stability in stock (DMSO) and plasma is still ongoing.

4. Clinical application

The recommended dose of dabrafenib is 150 mg bi daily and of trametinib 2 mg once daily. To test the applicability of this assay dabrafenib and trametinib concentrations were determined in 59 patient samples of patients that are treated with one or both drugs. The results are shown in Fig. 5. Almost all values are within the validated range of 50–5000 ng/mL for dabrafenib and 0.5–50 ng/mL for trametinib. Only one patient sample of trametinib had a value below the LLOQ, but this patient stopped therapy. These results demonstrate the applicability of this assay for clinical pharmacokinetic studies including TDM.

5. Conclusion

A sensitive LC–MS/MS assay for the quantification of dabrafenib and trametinib in human plasma and urine was developed and validated. The validated assay ranges from 50 to 5000 ng/mL for dabrafenib and 0.5–50 ng/mL for trametinib were linear with correlation coefficient (r^2) of 0.996 or better. Dilution integrity experiments show that samples can be diluted 10-fold in control matrix prior to analysis. The extended concentration range for dabrafenib is therefore from 50 to 25000 ng/mL and for trametinib, from 0.5 to 250 ng/mL. Dabrafenib is degraded into at least 5 degradation products under the influence of light and therefore amber colored vials should be used for dabrafenib containing samples. This assay is considered fit to support clinical pharmacologic studies of dabrafenib and trametinib.

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