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An UHPLC-MS/MS method for quantification of the CDK4/6 inhibitor abemaciclib in human serum

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ABSTRACT

Background: Abemaciclib is a new oral targeted treatment option for patients with advanced breast cancer. The emerging field of oral antitumor therapeutics presents challenges for both patients and healthcare teams; non-adherence and high inter-individual pharmacokinetic variability can influence response rates.
Methods: For monitoring abemaciclib in human sera, a rapid novel ultra-high-performance liquid chromatography-tandem mass spectrometry method was developed and fully validated. Sample preparation was based on a protein precipitation step followed by on-line solid phase extraction. Chromatographic separation was achieved using a biphenyl column and the isotope labeled standard abemaciclib-d_8 was used for quantification.
Results: The method showed linearity over a wide calibration range from 20.0 to 2500 ng/mL. With accuracies and precisions of ≤13.9% and ≤4.42%, respectively, the validation results were within the criteria of acceptance. The fitness of the method was tested by monitoring abemaciclib levels under compassionate use for a single individual.
Conclusions: The novelty of the presented two dimensional isotope dilution UHPLC-MS/MS method is in the semi-automated sample preparation, which results in negligible matrix effects, thereby allowing the introduction of abemaciclib into robust routine therapeutic drug monitoring (TDM). This method provides an efficient tool to verify the usefulness of personalized anticancer therapy in clinical practice.

Introduction

Since 2000, the FDA has approved more than 80 anticancer drugs of which a substantial proportion (10–15%) are targeted breast cancer agents [1,2]. The main mechanism of action of many antitumor therapeutics is the inhibition of protein kinases [3]. With about 2.3 million (24.5%) new cases per year, breast cancer is by far the most common cancer among women worldwide [4]. In Germany, there are about 70,000 breast cancer cases annually [5]. Preclinical and clinical data indicate that cyclin-dependent kinase 4 (CDK4)/CDK6 inhibitors are promising breast cancer drugs [6–8]. As of now, three compounds, palbociclib, ribociclib, and abemaciclib have been approved by the FDA and EMA. Abemaciclib is the most recent addition to the field of CDK4/6 inhibitors and was licensed by the FDA and EMA in 2017 and 2018, respectively [3,9,10]. Abemaciclib is a kinase inhibitor selective for CDK4/6 with antitumor activity in estrogen receptor-positive (ER+) breast cancer cells. These kinases are activated when binding to D-cyclines. Cyclin D1 and CDK4/6 promote phosphorylation of the retinoblastoma protein (Rb), cell cycle progression and cell proliferation in ER + breast cancer cell lines. Continuous exposure to abemaciclib can lead to inhibition of Rb phosphorylation and can block the progression from the G1 to the S phase of the cell cycle, resulting in senescence and apoptosis [6,9].

Due to the self-administration of oral tumor therapeutics (OTT), this type of treatment offers patients a more convenient option of this complex therapy. However, this emerging form of treatment poses considerable challenges for both patients and healthcare professionals [11] with rates of inadequate oral chemotherapy use reaching 16% to 100% [12,13]. Generally, a fixed dosing regimen is used, with the recommended dose for monotherapy being 200 mg of abemaciclib twice daily, which must be adjusted depending on side effects [14]. The occurrence of various side effects of abemaciclib (e.g., diarrhea, infections, neutropenia, anaemia) combined with pharmacokinetic interactions (cytochrome P450 (CYP) 3A4 inducers or inhibitors in polypharmacy) can influence therapeutic success. These challenges, and the recommendation of abemaciclib trough concentrations of 200 ng/mL to achieve sustained cell cycle arrest, indicate a possible proposal for individualized dosing and therapeutic drug monitoring (TDM)

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[9,15–18]. Additionally, more thorough pharmacokinetic and pharmacodynamic data are required for the exposure–response and the exposure-toxicity relationship of many OTT [16].

However, there is a lack of existing methods that address routine TDM of abemaciclib in serum samples. There are only a few methods, all of which are based on LC-MS/MS techniques, that describe the analyses of the CDK4/6 inhibitor abemaciclib in mouse and/or human plasma. The published methods are research methods based solely on manual sample preparation, and some are not directly applicable to human material due to its narrow calibration range [10,19–21].

Therefore, the main objective of the present study was to make TDM for abemaciclib more broadly available, and to develop a robust two-dimensional (2D) semi-automated isotope dilution (ID) UHPLC-MS/MS-method suitable for routine use for the reliable quantification of abemaciclib in human sera.

Materials and methods

Chemicals and reagents

Water, methanol, and acetonitrile (LC-MS grade) were obtained from J.T. Baker (Jackson, TN, USA). Formic acid was purchased from Merck (Darmstadt, Germany). Abemaciclib (purity 99.8%) and the internal standard abemaciclib-d_8 (purity 98.0%) were obtained from Alsachim (Illkirch, France). Drug-free serum (serum pool) was obtained from the blood donation center of the Bavarian Red Cross (Munich, Germany).

Patient samples

For method validation, anonymized and leftover serum and plasma samples from polypharmacized intensive care unit (ICU) and normal ward patients were used. The fitness of the developed method was tested by monitoring abemaciclib levels in an individual treated with abemaciclib under a compassionate use authorization. The patient provided written informed consent for participation in this study. Institutional Review Board ruled that approval was not required for this study. Serum samples (n = 20) were collected immediately prior to the scheduled drug intake (150 mg abemaciclib every 12 h), and serum (n = 7) and plasma (n = 7) samples were taken at time 0 and then every 2 h over a period of 12 hrs. Pharmacokinetics were calculated for both serum and plasma to obtain the area under the curve over the period of 12 hrs ($AUC_{0\text{-}12h}$) and the times (T_{min}/T_{max}) at which the minimum and maximum concentrations (C_{min} / C_{max}) were achieved [9,14]. Microsoft Excel was used to calculate AUC. Serum and plasma samples were stored at −80 °C for no longer than three weeks before abemaciclib concentrations were determined.

Calibrators, quality control samples, and internal standard solution

Standard stock solution of abemaciclib and abemaciclib-d_8 were prepared at a concentration of 1 mg/mL in methanol. Separate working solutions for calibrators and quality control samples of 40 μg/mL abemaciclib in methanol–water (33:67, v/v) were prepared fresh daily. Serial dilution of each working solution with blank serum resulted in seven calibrators (cal 1, 20.0 ng/mL; cal 2, 50.0 ng/mL; cal 3, 100 ng/mL; cal 4, 250 ng/mL; cal 5, 500 ng/mL; cal 6, 1000 ng/mL; cal 7, 2500 ng/mL) and four quality controls (QC A, 20.0 ng/mL; QC B, 50.0 ng/mL; QC C, 800 ng/mL; QC D, 2000 ng/mL). The blank and zero sample (cal 0) were directly prepared from drug-free serum.

The internal standard working solution of abemaciclib-d_8 was prepared from the stock solution with a concentration of 0.10 μg/mL in acetonitrile. Drug-free serum, all stock and working solutions, calibrators, and QCs were aliquoted and stably stored at −20 °C.

Sample preparation

For protein precipitation, 200 μL of the internal standard working solution (abemaciclib-d_8 0.10 μg/mL) were added to 50 μL of serum/plasma samples (blank, calibrator, QC, patient sample) in 1.5 mL polypropylene cups (Eppendorf, Hamburg, Germany) and thoroughly mixed for 30 s. After centrifugation at 15,000 g for 5 min at 20 °C (Centrifuge 5414 D, Eppendorf), 50 μL of the supernatant were diluted with 950 μL acetonitrile–water (30:70, v/v) and mixed for another 5 s.

2D-ID-UHPLC and MS/MS parameters

Analysis of the samples was conducted with a 2D Acquity UHPLC system consisting of an autosampler, two binary pumps, a switching valve, and a column oven interfaced with a triple quadrupole mass spectrometer Xevo TQ-S (Waters, Milford, MA, USA).

2D-UHPLC conditions

The 2D-UHPLC system protocol included the following steps: 1) on-line sample clean-up, 2) elution of the analyte to the analytical column, and 3) equilibration of the on-line and analytical column.

At the starting conditions with valve position A, pump 1 loaded the sample (injection volume 3 μL in partial loop mode) onto the on-line solid phase extraction column (SPE) (Oasis HLB Direct Connect HP column, 30 × 2.1 mm, 20 μm; Waters). After 0.6 min the switching valve changed to position B and the extracted sample was eluted in backflush-mode onto the analytical column by pump 2. For chromatographic separation a Raptor Biphenyl column (50 × 2.1 mm, 2.7 μm; Restek, Bad Homburg, Germany) equipped with a Raptor Biphenyl EXP guard column (5 × 2.1 mm, 2.7 μm; Restek) that was kept at 50 °C was used. For washing and re-equilibration of the on-line SPE, the switching valve changed back to position A after 1.1 min. The mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol; the weak and strong wash solvents were methanol–water (1:10, v/v) and 0.1% formic acid in acetonitrile, respectively. The total run time was 3.5 min. A switching valve allowed elution into the MS from 1.1 to 2.5 min of the UHPLC program. The switching valve, gradient, and flow rate conditions are shown in Fig. 1 and Table 1.

MS/MS conditions

Abemaciclib and abemaciclib-d_8 were analyzed in positive electrospray ionization mode. Two mass transitions as quantifier and qualifier were recorded by multiple reaction monitoring (MRM). To optimize the MS parameters for the analyte and internal standard, post-column infusion was performed with the pure standard solution.

The ion source parameters were set as follows: source temperature, 150 °C; desolvation temperature, 450 °C; desolvation gas flow, 900 L/Hr; cone gas flow, 150 L/Hr; capillary voltage, 3.5 kV; cone voltage, 25 V. The MS parameters, including precursor and product ions (quantifier and qualifier), collision energies (CE), retention, dwell times and ion ratios for abemaciclib and the corresponding internal standard, are shown in Table 2.

Samples were quantified with the TargetLynx 4.1 software (Waters) using the following settings: polynome type, linear; origin, excluded; weighting function, $1/x^2$.

Method validation

The requirements of the European Medicines Agency (EMA) Guideline on bioanalytical method validation were set as the basis for the method validation [22].

Selectivity and carry-over

The selectivity of the method was tested analyzing 20 different leftover fully anonymized serum and plasma samples from ICU and normal ward patients. To test for carry-over, blank samples were

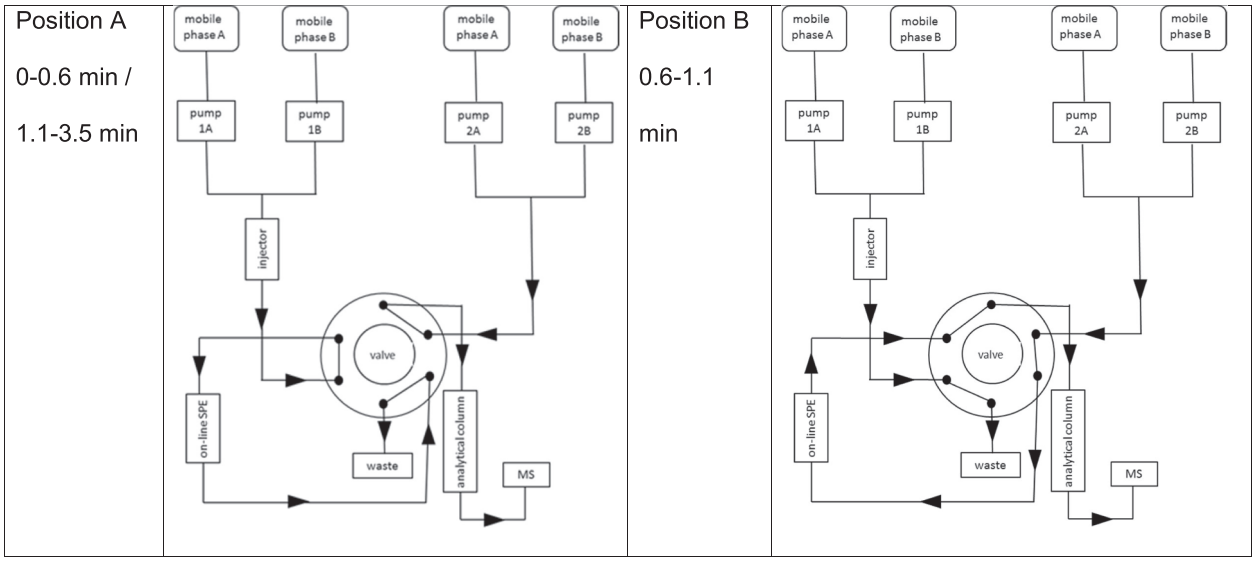

Fig. 1. 2D-UHPLC: On-line solid phase extraction system in position A and position B.

Table 1
Gradient and flow rate conditions of pump 1 and pump 2.

Pump 1				Pump 2			
Time [min]	Flow rate [mL/min]	A [%]	B [%]	Time [min]	Flow rate [mL/min]	A [%]	B [%]
0	1.5	90	10	0	0.5	50	50
0.5	1.5	90	10	0.8	0.5	50	50
0.6	0.1	90	10	1.8	0.5	0	100
0.9	0.1	0	100	2.5	0.5	0	100
1.0	0.1	0	100	2.6	0.5	50	50
1.1	1.5	0	100	3.5	0.5	50	50
2.1	1.5	0	100				
2.2	1.5	90	10				
3.5	1.5	90	10				

injected after the highest calibrator. The ion ratio of quantifier to qualifier of the analyte and the corresponding internal standard was monitored. Due to identical product ions and retention times of analyte and internal standard (Table 2), the method was additionally tested for crosstalk [23–25]. Crosstalk was examined by observing the signal of the internal standard even though only the analyte abemaciclib was injected (and vice versa).

Accuracy and precision

Within-run accuracy and precision was examined by five individual analysis of all four QC samples (n = 5). Between-run accuracy and precision was performed by analyzing five independent sample series on five different days (n = 5). One series included following samples that were prepared fresh daily: blank sample (without analyte and internal standard), zero sample (without analyte but added internal standard), calibrators 1–7, and QC A-D. The back-calculated concentrations of the calibration standards were also determined over five days (n = 5). Precision was expressed as the coefficient of variation (CV) and was calculated for every calibration and QC level. The deviation of the calculated value from the nominal value indicated the accuracy that was determined for each concentration level.

Dilution integrity

Blank samples (n = 5) were spiked above the upper limit of quantification (4000 ng/mL) and diluted with drug-free serum (1:5 to 800 ng/mL and 1:10 to 400 ng/mL, v/v) before further processing. To evaluate the dilution integrity, the measured concentrations were compared to the expected concentrations.

Stability

QC samples (level B and level D) were each used as triplicates to evaluate the stability of abemaciclib. Processed samples were stored at 8 °C for 24 h in the autosampler. For benchtop stability, unprocessed QCs were kept up to 4 h at ambient temperature. Three repeated freeze and thaw cycles (-20 °C to ambient temperature with freeze time ≥ 24 h) were investigated with unprocessed samples. Long term stability of the unprocessed samples was evaluated at −20 °C for up to 4 weeks. For every storage condition, the mean obtained values were compared to the nominal values of the QC samples.

Matrix effects

For evaluating the matrix effect, six different blank serum samples including two lipemic, two icteric, and two hemolytic patient samples were spiked in triplicate with abemaciclib at two different concentrations (QC B and QC D) and internal standard, respectively. The measured mean concentrations of the spiked samples were compared to the nominal concentrations as suggested in the ICH guideline M10 on bioanalytical method validation (EMA) [26].

Additionally, a post column infusion (PCI) experiment according to Bonfiglio et al. [27] was performed. Extracted samples and a mixture of acetonitrile–water (30:70, v/v) were injected while a solution of

Table 2
MS parameter for abemaciclib and abemaciclib-d_8.

Analyte	Molecular weight [g/mol]	Retention time [min]	Precursor Ion [m/z]	Product Ion [m/z]	CE [eV]	Dwell time [ms]
Abemaciclib	506.59	1.39	507.4	393.3[a]	20	133
			507.4	245.2[b]	45	
Abemaciclib-d_8	514.66	1.39	515.4	393.3[a]	20	
			515.4	245.2[b]	45	

CE, collision energy; a, quantifier; b, qualifier.

abemaciclib (0.01 μg/mL) in water-methanol (1:1, v/v) was continuously infused into the MS (flow rate 20 μL/min). The respective chromatograms were compared to evaluate qualitative matrix effects.

Results

Method development report

The molecular structure of abemaciclib (2-Pyrimidinamine, N-[5-[(4-ethyl-1-piperazinyl)methyl]-2-pyridinyl]-5-fluoro-4-[4fluoro-2-methyl-1-(1-methylethyl)-1H-benzimidazol-6-yl]) (Fig. 2) includes heterocyclic aromatic amines [14]. Therefore, a biphenyl column was tested, which showed good overall chromatographic performance. A few LC-MS/MS methods or chromatographic conditions have been published for the analysis of abemaciclib: C18 columns with alkaline or acidified mobile phases have been described by Martinéz-Chávez et al. [10,19], Wickremsinhe et al. [20], and Kadi et al. [21], respectively, with protein precipitation performed for sample cleanup. The herein developed sample preparation consisted of a manual protein precipitation step followed by an automated on-line sample clean-up using a hydrophilic-lipophilic balanced reversed-phase sorbent. Since carry-over is a frequently described problem for abemaciclib and similar analytes, acidified mobile phases (A 0.1% formic acid in water and B 0.1% formic acid in methanol) were also used for the on-line approach in addition to acidified strong wash solvent (0.1% acetonitrile) [10,28]. A representative 2D-ID-UHPLC-MS/MS chromatogram of an authentic serum sample of abemaciclib (1.39 min; 49.3 ng/mL) with semi-automated sample preparation is shown in Fig. 3.

Method validation

Selectivity and carry-over

To demonstrate selectivity and carry-over, the response should be ≤ 20% of the LLOQ for analytes and ≤ 5% for internal standards. No interference was observed for abemaciclib-d8 and the response of abemaciclib did not exceed 15.1% of the LLOQ (20 ng/mL). The carry-over for the internal standard abemaciclib-d8 was ≤ 1.93%. For the analyte abemaciclib, the carry-over was ≤ 1.27% with respect to calibrator 7 (2500 ng/mL), which was equivalent to ≤ 170% of the LLOQ due to the wide calibration range. This result exceeded the requirement of the EMA guideline [22], hence two blank injections are highly recommended after a high-level sample above 500 ng/mL. With a second blank injection, carry-over was reduced to < 20% of the LLOQ for samples with extremely high concentrations. The mean quantifier to qualifier ion ratio of abemaciclib and abemaciclib-d_8 was 5.4 ± 0.8 over the entire validation period. A signal from the internal standard upon injection of the analyte (and vice versa) would indicate crosstalk. Despite the same product ions and retention times of analyte and internal standard no crosstalk was observed [23–25].

Accuracy and precision

Accuracy and precision for the back-calculated concentrations (measured value vs. nominal value) of the calibration standards and the QCs including the LLOQ were within the limits of the EMA guideline (±15% and ± 20% for the LLOQ) [22]. The summarized results for within-run and between-run accuracy and precision for abemaciclib are shown in Table 3. The deviations of the back-calculated concentrations of the calibration standards did not exceed 8.64 ± 6.35%. Linearity with $r^2 \geq 0.9976$ was given over a wide calibration range from 20 ng/mL to 2500 ng/mL abemaciclib. The slope of the calibration curve was 1.179 ± 0.075 and the intercept was 2.778 ± 3.596. The signal to noise ratio of the LLOQ (20 ng/mL) was at least 10:1.

Dilution integrity

For both dilution factors (1:5 and 1:10, v/v), accuracy and precision of the dilution integrity was ≤5.55% and 2.10%, respectively, and was below the set criteria of 15% for accuracy and precision [22].

Stability

To demonstrate stability the mean concentrations should be within ± 15% of the nominal concentrations. Benchtop stability was tested and given up to 4 h with deviations ≤ 9.84% from the nominal values. Processed samples were found to be stable at 8 °C in the autosampler for 24 h with deviations ≤ 11.9% from the nominal value. Abemaciclib showed a long term-stability for 28 days at −20 °C and also remained stable for three repeated freeze-and-thaw-cycles with maximum deviations between −13.2% and 5.05%, respectively.

Matrix effects

Because of semi-automated sample preparation, matrix effect according to the ICH guideline M10 on bioanalytical method validation (EMA) [26] was conducted with fully anonymized icteric, lipemic, and hemolytic serum samples from ICU patients. The acceptability ranges for accuracy and precision were defined as a maximum of 15% of the nominal values [26]. With accuracy ≤ 8.47% and precision ≤ 5.11%, the matrix effects were within the required range and no difference between these different types of samples were found. The two PCI chromatograms resulting from injecting a blank sample and solvent were nearly congruent. Any difference of the two chromatograms, especially at the retention time of abemaciclib, would indicate matrix effects. Thus, negligible matrix effects were also confirmed by the PCI experiment (Fig. 4.) according to Bonfiglio et al. [27].

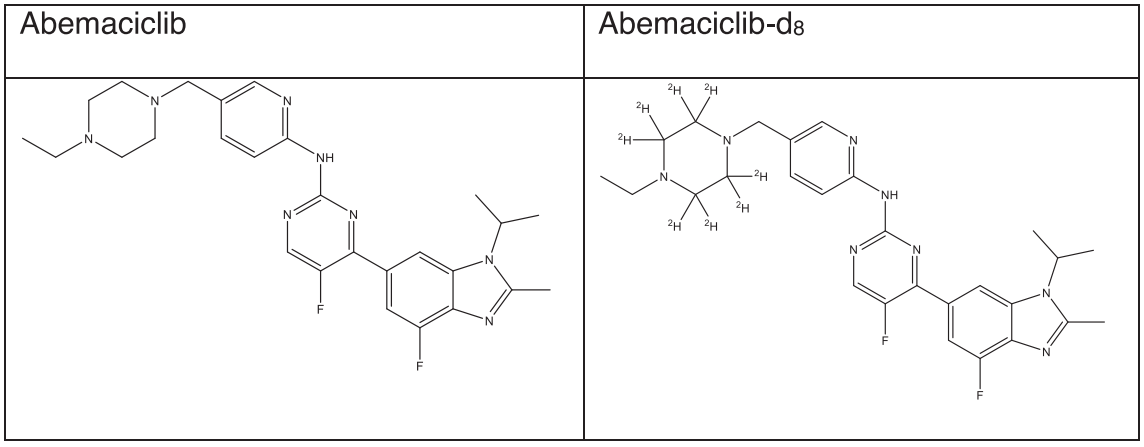

Fig. 2. Molecular structure of abemaciclib and abemaciclib-d_8.

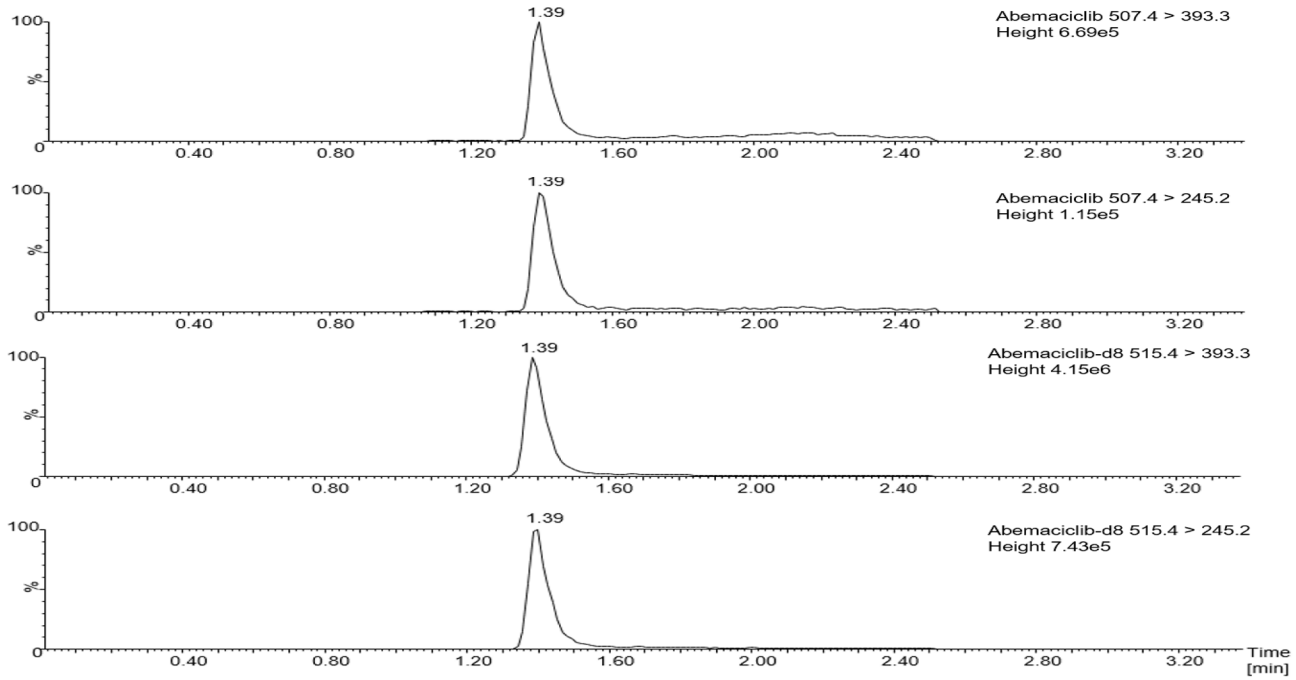

Fig. 3. 2D-UHPLC-MS/MS chromatogram of abemaciclib and the internal standard abemaciclib-d8 of an authentic serum sample (49.3 ng/mL abemaciclib).

Table 3
Summarized validation results for abemaciclib.

Abemaciclib Target concentration QC, [ng/mL]	QC A 20.0	QC B 50.0	QC C 800	QC D 2000
Accuracy within-run (n = 5), [%]	6.89	0.53	12.9	8.12
Precision within-run (n = 5), [%]	3.37	2.26	1.36	2.24
Accuracy between-run (n = 5), [%]	13.9	9.69	9.53	8.53
Precision between-run (n = 5), [%]	4.16	4.42	3.28	2.41
Accuracy autosampler stability (n = 3), 24 h, 8 °C, [%]	–	11.9	–	0.66
Accucary benchtop stability (n = 3), 4 h, 23 °C, [%]	–	1.18	–	9.84
Accuracy freeze–thaw-cycles (n = 3), 3 cycles, [%]	–	5.05	–	−13.2
Accuracy long term-stability (n = 3), 28 days, −20 °C, [%]	–	−6.29	–	−9.50
Accuracy matrix effect (n = 3), 6 lots, [%]	–	8.47	–	2.56
Precision matrix effect (n = 3), 6 lots, [%]	–	3.11	–	5.11
Accuracy dilution integrity (n = 5), 1:5 (v/v), [%]	5.55 ± 2.10			
1:10 (v/v), [%]	2.93 ± 1.92			

Fitness for purpose

As a proof-of-concept of the developed method, the blood from one patient was collected each time prior the scheduled drug intake (n = 20) and analyzed for abemaciclib. The collected samples contained between 21.8 ng/mL and 68.4 ng/mL of abemaciclib. The serum and plasma concentration–time profile over 12 h following regular 12 h dosing with 150 mg abemaciclib is shown in Fig. 5. The pharmacokinetic parameters of abemaciclib in serum were an $AUC_{0\text{-}12h}$ of 673 ng*h/mL, a T_{min} at 12 h with a C_{min} of 42.3 ng/mL, and a T_{max} at 4 h with a C_{max} of 66.9 ng/mL. In comparison, the pharmacokinetic parameters in plasma showed no significant difference, with an $AUC_{0\text{-}12h}$ of 638 ng*h/mL, a T_{min} at 12 h with a C_{min} of 38.9 ng/mL, and a T_{max} at 6 h with a C_{max} of 62.1 ng/mL.

Discussion

We developed and fully validated a rapid and robust 2D-ID-UHPLC-MS/MS method for abemaciclib based on semi-automated sample preparation. We decided to set the calibration range for abemaciclib to a factor of 10 below and above the recommended trough value of 200 ng/mL (calibration from 20 ng/mL to 2500 ng/mL) [17,18]. The method showed an accuracy and precision of all QCs (including LLOQ level) ≤ 13.9% and ≤ 4.42%, respectively. During the extensive validation period, the method proved to be robust and rugged in terms of analytical selectivity, linearity, accuracy, precision, matrix effects, and stability. For more than one year, the described method has been used and tested for research purposes. Reliable results from this testing, in combination with no outliers or malfunctions, supports introduction of the assay as part of routine TDM. In our laboratory, 2D-chromatography has been successfully used for years, especially for the analysis of a large number of immunosuppressant samples. The method presented here is also based on a semi-automated sample preparation with on-line SPE, which has not yet been described for quantification of abemaciclib.

Previous methods analyzing CDK4/6 inhibitors like abemaciclib, palbociclib, or ribociclib used C8-, C18-, and amide columns and often dealt with tailing peak shapes or carry-over [10,28–30]. The biphenyl stationary phase used here was a well-suited chromatographic tool for analyzing abemaciclib with good peak shape. With the described method, we analyzed authentic serum levels and show so far rarely published kinetic data of abemaciclib in serum and plasma. No significant difference was observed between abemaciclib in serum or plasma, indicating that both sample matrices are suitable and comparable for the determination of abemaciclib levels. The mean observed values of abemaciclib were dose-dependent between 34.0 and 298 ng/mL when administered orally twice daily, with $AUC_{0\text{-}12h}$ reported in the steady state from 546 to 3000 ng*h/mL [31]. Tate et al. [18] also reported dose-dependent, but varying, abemaciclib plasma levels in the range of 1–2000 ng/mL. Trough plasma concentrations of 200 ng/mL were observed and recommended to achieve sustained cell-cycle arrest [17,18]. Despite the high variability of abemaciclib exposure in patients, a fixed dosing paradigm is common practice [16,18]. In the medical context, OTT self-management in the home environment is a challenge

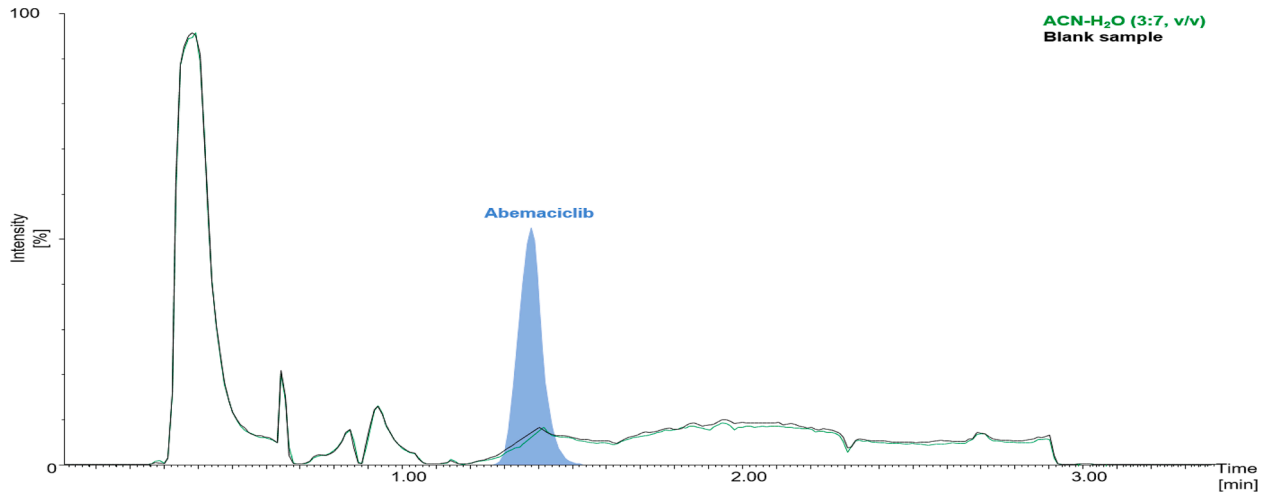

Fig. 4. Post-column infusion chromatogram of abemaciclib (green, ACN-H20 (3:7, v/v); black, blank sample). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

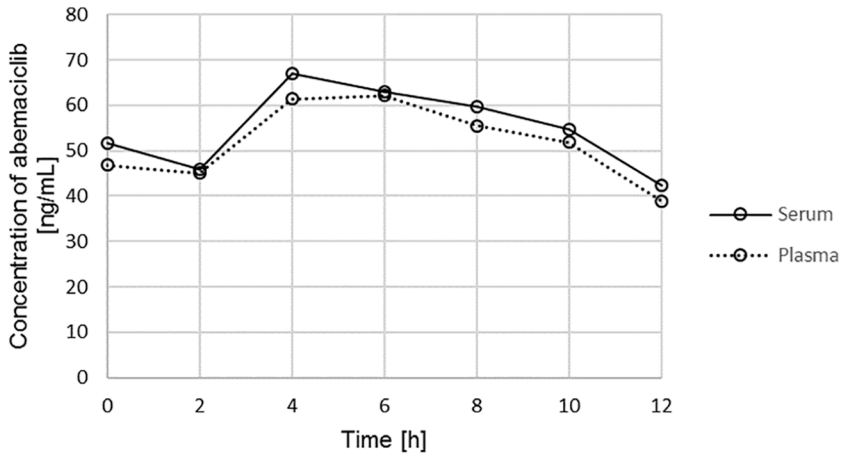

Fig. 5. Human serum and plasma concentration–time profile of abemaciclib over 12 h following regular 12 h dosing with 150 mg abemaciclib. Serum (n = 7) and plasma (n = 7) samples from a single patient were collected in direct succession every 2 h for pharmacokinetic assessment.

for the health care system, health care professionals, and patients. In general, treatment with abemaciclib is monitored by personnel experienced in oncology, but safer practices need to be developed to avoid medication errors [32]. Due to various side effects and adverse reactions of abemaciclib, like neutropenia, anemia, and diarrhea, dose modifications might be necessary [14]. Polypharmacy leads to challenges to identify and eliminate drug interactions, with a special focus on CYP3A4 inhibitors (e.g., ketoconazole, grapefruit products), which can increase the AUC of abemaciclib by up to 16-fold [9,14]. Comprehensive pharmaceutical care and patient education programs for oral cancer treatments typically result in high patient satisfaction rates and improvements in safety and efficiency [33,34]. Also, with regard to the "drug-omic" approach [35], TDM of abemaciclib can provide an additional solution to increase drug therapy safety.

We believe that, based on this convenient method, it is possible to set up a routine TDM program, especially to address drug therapy safety, treatment success, and patient adherence. In the future, the presented semi-automated 2D-ID-UHPLC-MS/MS method may be adapted and extended to include further CDK4/6 inhibitors and their active metabolites.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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