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Short communication

Development and validation of a liquid chromatography-tandem mass spectrometry assay for nine oral anticancer drugs in human plasma

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ABSTRACT

A liquid chromatography-tandem mass spectrometry assay was developed and validated for the nine oral anticancer agents alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, palbociclib, ribociclib, vismodegib and vorinostat in order to support therapeutic drug monitoring (TDM). The assay was based on reversed-phase chromatography coupled with tandem mass spectrometry operating in the positive ion mode. The assay was validated based on the guidelines on bioanalytical methods by the US Food and Drug Administration and European Medicines Agency. The method was validated over a linear range of 10–200 ng/mL for alectinib, lenvatinib, nintedanib and vismodegib; 50–1000 ng/mL for cobimetinib and palbociclib; 100–2000 ng/mL for osimertinib; 5.00–100 ng/mL for ribociclib; 25–500 ng/mL for vorinostat. Intra-assay and inter-assay bias was within ±20% for all analytes at the lower limit of quantification and within ±15% at remaining concentrations. Stability experiments showed that osimertinib is unstable in the biomatrix and should be shipped on dry-ice and stored at -20 °C until analysis. All other compounds were stable in the biomatrix. The described TDM method was successfully validated and applied for TDM in patients treated with these KIs.

1. Introduction

The number of oral anticancer agents approved for the treatment of various malignancies is rapidly expanding. These anticancer drugs are designed to interact with a specific target expressed by malignant cells. For many of these kinase inhibitors (KIs), exposure-response and exposure-toxicity relationships have already been examined during the development phase, mainly in phase II and III clinical trials. During these clinical trials, only fixed doses are explored, resulting in fixed dose treatment strategies when the drug is registered and used in clinical practice. It has however been shown that drug exposure may considerable vary between patients. Patients with a high exposure may develop excessive toxicity while patients with low exposures may be at risk of treatment failure [1].

By using Therapeutic Drug Monitoring (TDM), treatment outcomes can be optimized. Based on the quantification of individual plasma concentrations and interpretation of these concentrations with respect to proposed target concentrations, dose individualizations can be applied [2].

A TDM assay was developed and validated for the concurrent quantification of the new KIs alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, palbociclib, ribociclib, vismodegib and vorinostat. These drugs are registered or under investigation for various oncological indications. Several bioanalytical methods using liquid chromatography coupled to mass spectrometry have been described for the quantification of alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, vismodegib and vorinostat in human plasma, either alone or in combination assays [3–8]. For TDM purposes, a rapid, robust and efficient quantification is preferable, which we describe here. Most importantly, our assay enables simultaneous quantification of the plasma concentrations

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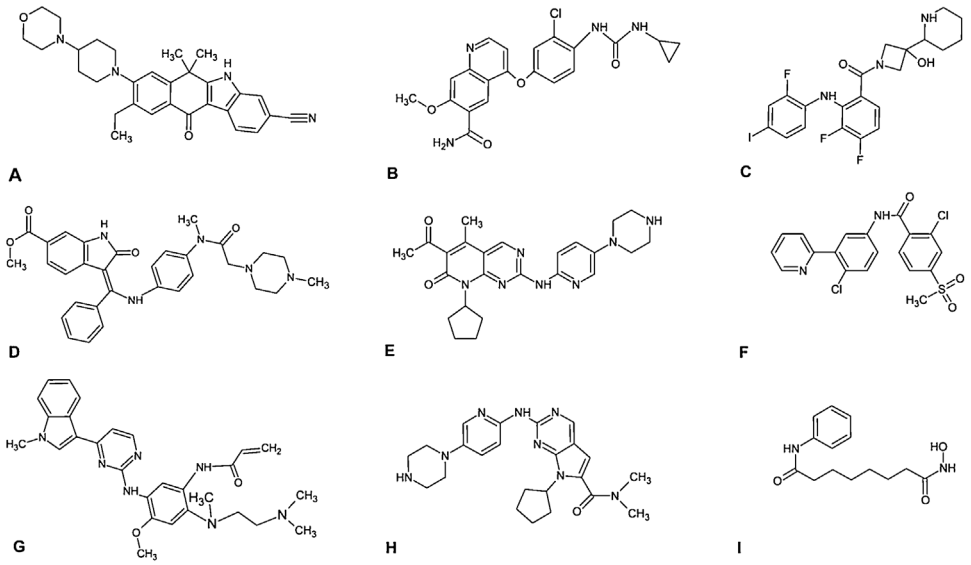

Fig. 1. Molecular structure of alectinib (A), lenvatinib (B), cobimetinib (C), nintedanib (D), palbociclib (E), vismodegib (F), osimertinib (G), ribociclib (H) and vorinostat (I).

of patients receiving one of these KIs. In addition, we demonstrate that the assay is applicable in clinical routine care.

2. Material and methods

2.1. Chemicals

Alectinib, $^{2}H_8$-alectinib, lenvatinib, $^{2}H_5$-lenvatinib, nintedanib, $^{13}C,^{2}H_3$-nintedanib, osimertinib, $^{13}C,^{2}H_3$-osimertinib, palbociclib, $^{2}H_8$-palbociclib, ribociclib, $^{2}H_6$-ribociclib, vismodegib, $^{13}C_7$-vismodegib, $^{13}C_6$-vorinostat and $^{13}C_6$-cobimetinib were purchased from Alsachim (Illkirch Graffenstaden, France). Vorinostat and ammonium bicarbonate were bought at Sigma Aldrich (Zwijndrecht, the Netherlands) and cobimetinib was bought at Selleckchem (Houston, USA). Acetonitrile, methanol and water (all Supra-Gradient grade) were from Biosolve Ltd (Valkenswaard, The Netherlands). K_2EDTA plasma was obtained from Bioreclamation LLC (Hicksville, USA). The chemical structures of the analytes are depicted in Fig. 1.

2.2. Stock solutions and working solutions

Stock solutions were prepared at a concentration of 1 mg/mL in DMSO for cobimetinib, lenvatinib, nintedanib, osimertinib, ribociclib, vismodegib and vorinostat. The stock solution containing alectinib was prepared at a concentration of 0.5 mg/mL in DMSO. Since palbociclib is only soluble in acetic aqueous solutions, 0.1% formic acid in water was used as a solvent for the palbociclib stock solution. The stock solutions were diluted with methanol to obtain working solutions. For alectinib, lenvatinib, nintedanib and vismodegib working solutions were prepared at concentrations of 200, 500, 2000, 4000 and 20,000 ng/mL, at concentrations of 1000, 2500, 10,000 and 20,000 ng/mL for cobimetinib and palbociclib, at concentrations of 100, 250, 1000, 2000 and 10,000 ng/mL for ribociclib, at concentrations of 500, 1250, 5000 and 10,000 ng/mL for vorinostat and at concentrations of 2000, 5000, 20,000 and 40,000 ng/mL for osimertinib. Two independent stock solutions and working solutions were prepared for the preparation of calibration standards and quality control (QC) samples. Stock solutions for all internal standards (IS) were prepared at a concentration of 1 mg/mL in DMSO, except for $^{2}H_8$-palbociclib which was prepared in 0.1% formic acid in water and stored in amber-coloured 2.0-mL tubes. Subsequently, a mixture of the internal stock solutions was prepared and diluted with methanol at concentrations of 2500 and 25,000 ng/mL for $^{2}H_8$-alectinib, $^{13}C_6$-cobimetinib, $^{2}H_5$-lenvatinib, $^{13}C,^{2}H_3$-osimertinib, $^{2}H_8$-palbociclib and $^{13}C_7$-vismodegib and at concentrations of 250, 2500 and 25,000 for $^{13}C,^{2}H_3$-nintedanib and $^{2}H_6$-ribociclib. All stock- and working solutions were stored at -20°C.

2.3. Calibration standards, quality control samples

1900 μL blank human K_2EDTA plasma was spiked by 100 μL of the calibration standard working solution. The QC samples were prepared by spiking 100 μL of the separately prepared working solution to 1900 μL blank human K_2EDTA plasma to obtain the final QC low, QC mid and QC high concentrations. Both calibrators and QC samples were subsequently stored in aliquots of 50 μL and stored at −70 °C. Back-calculated concentrations of the calibrators were used for the determinations of the linearity of the calibration model, using the reciprocal of the squared concentrations ($1/x^2$) as the weighting factor. For all analytes, linearity was concluded for the previously described validation concentration ranges. All other requirements were also in accordance with the guidelines, for all nine analytes (≥75% of the calibrators within ±15% (±20% for the LLOQ) of the nominal concentrations and analyte response at LLOQ level was ≥ 5 times the response compared to the blank response).

2.4. Sample preparation

Samples were collected by venipuncture in the clinic. Directly after withdrawal, samples were centrifuged for 10 min at 2000*g* at 4 °C. After centrifugation, plasma was isolated and stored at -20 °C. Prior to processing, each sample was thawed and 50 μL was aliquoted in 1.5 mL amber-coloured tubes. Samples were spiked with 20 μL of IS working solution and 150 μL acetonitrile was added. Afterwards, samples were vortex-mixed for 10 s and centrifuged for 10 min at 15,000 rpm. 100 μL supernatant was added to an amber-coloured autosampler vial with insert that contained 100 μL 10 mM ammonium bicarbonate in water. The final extract was vortex-mixed for 10 s and stored at 2–8 °C until analysis.

Table 1
Mass spectrometric settings for the analytes and their internal standards.

Parameter	
Run duration	4.00 min
Ion spray voltage	4000 V
Nebulizer gas	30 au
Collision gas	9 au
Curtain gas	30 au
Turbo gas	40 au
Temperature	500 °C
Dwell time	20 ms

Specific Parameters Analyte	MRM (Da)	Collision energy (V)	Collision exit potential (V)	Declustering potential (V)	Retention time (min)
Alectinib	483.1 → 396.1	41	54	71	1.62
$^{2}H_8$-alectinib	491.1 → 396.1	41	54	71	1.61
Cobimetinib	532.1 → 249.0	45	22	171	1.54
$^{13}C_6$-cobimetinib	538.1 → 255.0	45	22	171	1.54
Lenvatinib	428.1 → 371.0	43	52	61	1.22
$^{2}H_5$-lenvatinib	433.1 → 371.0	43	52	61	1.21
Nintedanib	540.3 → 113.1	33	14	136	1.42
$^{13}C,^{2}H_3$-nintedanib	544.3 → 117.1	33	14	136	1.41
Osimertinib	500.2 → 72.1	47	20	120	1.69
$^{13}C,^{2}H_3$-osimertinib	504.2 → 72.1	47	20	120	1.69
Palbociclib	448.2 → 380.1	43	48	51	1.36
$^{2}H_8$-palbociclib	456.2 → 388.1	43	48	51	1.35
Ribociclib	435.2 → 322.1	49	30	181	1.34
$^{2}H_6$-ribociclib	441.2 → 322.1	49	30	181	1.23
Vismodegib	420.9 → 342.2	51	26	186	1.27
$^{13}C_7$-vismodegib	430.9 → 349.7	51	26	186	1.26
Vorinostat	265.2 → 232.0	15	30	51	1.07
$^{13}C_6$-vorinostat	270.2 → 237.0	15	30	51	1.07

2.5. Chromatographic equipment and conditions

A Waters Acquity I class UPLC system with binary pump, integrated degasser, column oven and I class autosampler were used (Waters, Milford, MS, USA). The temperature of the autosampler and column were kept at 8 °C and 40 °C, respectively. The highest selectivity was obtained by a basic eluent. Mobile phase A consisted of 10 mM ammonium bicarbonate (pH 10.5) in water and mobile phase B consisted of 10 mM ammonium bicarbonate (pH 10.5) in methanol-water (1:9, v/v). Gradient elution was applied at a flow of 0.25 mL/min through a Gemini C_{18} column (50 × 2.0 mm ID, 5.0 μm) with an additional Gemini C18 guard column (4 × 2.0 mm ID, 5.0 μm) (both Phenomenex, Torrance, CA, USA). The applied gradient program was based on a previously reported TDM-assay (40% B at 250 μL/min (0-0.1 min); 40–100% B at 250 μL/min (0.1–1 min); 100% B at 250 μL/min (1.0–3.0); 40% B at 500 μL/min (3.01–3.50 min); 40% B at 250 μL/min (3.51–4.0 min)). [9]

2.6. MS equipment and conditions

A QTRAP5500 triple quadrupole mass spectrometer (MS) equipped with a turbo ion spray interface, operating in positive ion mode was used (AB Sciex, Framingham, MA, USA). Multiple reaction monitoring (MRM) chromatograms were acquired and processed using Analyst™ software (AB Sciex, version 1.6.2). The MS operating parameters are summarized in Table 1 and Supplementary Fig. 1 shows chromatograms of QC lower limit of quantification (LLOQ) level and double blank samples.

2.7. Validation procedures

The assay was validated based on the FDA and EMA guidelines on bioanalytical method validation. [10,11]

3. Results and discussion

3.1. Accuracy and precision

In order to assess intra- and inter-assay accuracies and imprecisions, five replicated QC samples were analyzed in three consecutive runs at the LLOQ, midrange and high concentrations. The accuracy (bias) was determined in percentage difference between the mean measured concentration (per run for intra-assay bias and overall for inter-assay bias) and the nominal concentration and coefficient of variation (CV%) were used to assess the intra-run precision. Analysis of Variance (ANOVA) was used to assess inter-run precision. The inter- and intra-assay accuracy and imprecision were ≤20% for the LLOQ and ≤15% for midrange and high concentrations and thus within the acceptance criteria for all analytes. Details on the assay performance data are listed in Table 2.

3.2. Dilution integrity

High and variable concentrations in patient samples were expected for vismodegib and alectinib based on previously published pharmacokinetic studies. [12,13] To investigate the dilution integrity for these analytes, five replicates of both vismodegib (100-fold dilution) and alectinib (10-fold dilution) were prepared in blank human plasma. Bias was within ±15% and the CV% was ≤15% for both vismodegib and alectinib.

3.3. Carry-over

Two double blank samples were injected after an ULOQ sample, in order to determine carry-over. Interferences in the double blank sample should be ≤ 20% of the analyte peak area of the LLOQ sample and ≤ 5% of the peak area of the IS. For cobimetinib, osimertinib, palbociclib and vorinostat, eluting peaks with areas exceeding 20% of the LLOQ were observed in blank samples injected after ULOQ

Table 2
Assay performance data for the analysis of alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, palbociclib, ribociclib, vismodegib and vorinostat.

		Intra-assay		Inter-assay	
Analyte	Nominal concentration (ng/mL)	Bias (%)	CV%	Bias (%)	CV%
Alectinib	10	−0.1 to 6.4	7.9–15.9	3.7	-[a]
	100	−3.3 to 13.6	2.5–6.3	3.2	8.5
	200	5.0–10.3	1.6–12.2	7.4	-[a]
Cobimetinib	50	−13.4 to 8.8	4.6–10.2	−3.9	11.5
	500	−0.1 to 10.5	3.4–7.2	3.5	5.2
	1000	11.0–12.8	1.2–8.7	11.6	-[a]
Lenvatinib	10	1.7–8.8	10.1–16.1	5.8	-[a]
	100	−3.0 to 7.8	4.4–5.5	2.2	4.8
	200	8.7–13.7	4.4–11.9	11.0	-[a]
Nintedanib	10	−4.4 to 15.8	9.0–16.9	3.3	8.9
	100	−1.2 to 5.2	3.9–11.6	2.3	-[a]
	200	7.3–14.7	4.5–11.7	10.2	-[a]
Osimertinib	99.9	−15.3 to 9.2	3.7–4.3	0.2	13.3
	999	−13.5 to 5.0	1.6–4.4	−1.4	10.5
	2000	−8.8 to 7.8	1.3–2.4	2.2	9.3
Palbociclib	50.2	−3.2 to 10.5	2.9–9.6	5.2	6.2
	502	−5.1 to 9.8	2.0–10.2	2.4	6.8
	1000	3.5–14.4	3.5–14.4	8.6	3.5
Ribociclib	4.99	−8.8 to 2.2	3.7–8.3	−2.3	5.1
	49.9	0.3–13.9	2.4–8.6	9.1	6.5
	99.8	−1.2 to 8.8	4.3–5.6	3.4	4.4
Vismodegib	10	−2.1 to 12.5	4.0–10.6	7.3	6.9
	100	−1.8 to 14.0	8.2–11.1	5.2	6.3
	200	2.5–11.8	2.0–8.8	6.8	3.1
Vorinostat	25	−2.2 to 4.2	6.9–15.1	1.4	-[a]
	250	−12.7 to −2.4	5.1–10.5	−6.7	4.5
	501	−4.3 to 7.2	3.5–12.7	1.5	4.0

[a] Inter-run precision could not be calculated because there is no significant additional variation owing to the performance of the assay in different runs.

samples. Carry-over into the second double blank samples was around 20% of the LLOQ for osimertinib, ribociclib and vorinostat. Two blank samples were injected after ULOQ and QC high samples during the validation to control the carry-over. Clinical samples containing these drugs should not be grouped during analysis to circumvent impact of carry-over.

3.4. Specificity and selectivity

The selectivity of the method was determined by the analysis of LLOQ samples prepared in six different batches of blank human plasma. For at least 5 out of 6 batches, the mean measured concentrations at LLOQ level were within ±20% of the nominal concentrations for all analytes and interferences were ≤20% (≤5% for the IS) of the LLOQ areas in at least 5 of the 6 double blanks. The cross analyte/IS interferences were determined by separately spiking the analytes and IS to blank human plasma at their ULOQ. Cross-analyte interference was found for lenvatinib on the cobimetinib LLOQ signal (30%), cobimetinib on the nintedanib LLOQ signal (33%), nintedanib IS on the nintedanib LLOQ signal (22%) and for ribociclib IS on the ribociclib LLOQ signal (24%). Cross-analyte interference has no impact on the quantification of patient samples since lenvatinib, cobimetinib and nintedanib are not used concomitantly by patients and thus samples contain only one analyte. The calibration standards and QC samples do not contain LLOQ and ULOQ levels in the same sample and therefore the cross-analyte interferences from lenvatinib and cobimetinib are negligible. Furthermore, the concentrations of the nintedanib and ribociclib IS were lowered in order to minimize cross-interfering on patient samples. Cross-analyte interferences of co-eluting peaks were accepted for all other IS and concentrations at LLOQ level.

3.5. Matrix effect

Six batches of individual blank human plasma at low and high concentrations in singular were prepared to determine the matrix effect. For both the analyte and IS, the matrix factor (MF) was calculated for each matrix lot by calculating the ratio of the peak area in the presence of matrix to the peak area in absence of matrix (working solution of the analyte). Furthermore, the IS normalized MF was calculated by dividing the MF of the analyte by the MF of the IS. At both tested QC concentration levels the CV of the IS-normalized matrix factor from the 6 batches ranged from 0.83 to 0.98 and CV% were ≤ 15% for all analytes and were thus considered acceptable.

3.6. Stability

The analytes were considered stable in the matrix when 80–120% of the initial measured concentration was found for the LLOQ level and when 85–115% of the nominal concentration was found for the other concentration levels. For stock and working solutions acceptance criteria of 95%–105% were applied. Plasma samples were stable for at least up to 48 h at 20–25 °C when protected from light, 5 weeks at −20 °C, 18 weeks at −70 °C and after three freeze (−20 °C) -thaw (20–25 °C) cycles. The processed samples were stable for at least 48 h at 2–8 °C except for osimertinib. Additional stability experiments have been performed to assess the stability of osimertinib. These experiments showed that osimertinib is stable for 4 h at 20–25 °C, 6.5 h at 2–8 °C and 5 weeks at −20 °C. Similar results were observed during the validation of a previously reported LC–MS/MS assay for osimertinib. [14] Therefore, clinical samples containing osimertinib should be sent on dry-ice to the laboratory and stored at −20 °C until analysis. In addition, sample preparation and analysis time should be limited to 4 h. The stock and working solutions were stable at −20 °C for at least 8 and 5 months, respectively. Long-term stability at −70 °C is still ongoing.

3.7. Clinical application

As part of routine clinical care, K_2EDTA whole blood samples were collected from patients who were treated at our institute.

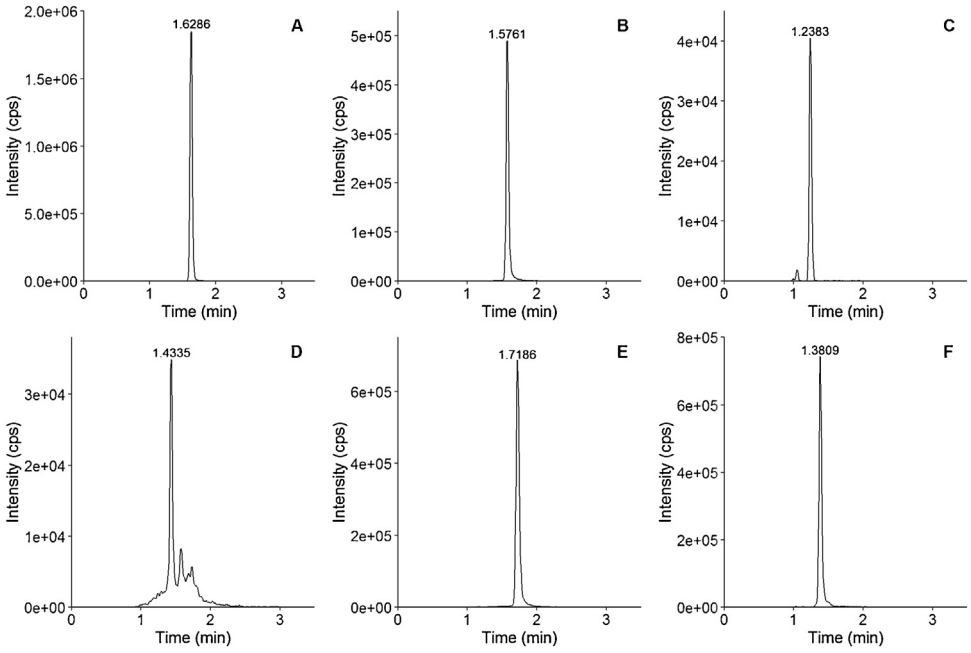

Fig. 2. Chromatograms of analyzed plasma samples collected from patients treated with alectinib (A, 793 ng/mL), cobimetinib (B, 253 ng/mL), lenvatinib (C, 91.1 ng/mL), nintedanib (D, 5.05 ng/mL), osimertinib (E, 331 ng/mL), palbociclib (F, 75.3 ng/mL).

Table 3
Plasma concentrations and calibration range of alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, palbociclib, ribociclib, vismodegib and vorinostat of patient treated with these drugs (n=10).

Analyte	Mean plasma concentration (ng/mL)	Range (ng/mL)	Calibration range (ng/mL)
Alectinib[a]	793	262 – 2520	10–200
Cobimetinib	253	69.7 – 649	50–1000
Lenvatinib	91.1	20.4 – 267	10 – 200
Nintedanib[b]	24.5	13.0 – 36.0	10 – 200
Osimertinib	331	123 – 798	100–2000
Palbociclib	90.3	54.2–186	50–1000
Ribociclib[c]	–	–	5 – 100
Vismodegib[c]	–	–	10–200
Vorinostat[c]	–	–	25–500

[a] Alectinib samples are diluted 10-fold prior to analysis.
[b] Only three clinical samples have been collected for nintedanib.
[c] No clinical samples have been collected yet for ribociclib, vismodegib and vorinostat.

Representative chromatograms and corresponding concentrations are depicted in Fig. 2. Mean measured concentrations and corresponding ranges are listed in Table 3. These results demonstrate the applicability of this assay for alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib or palbociclib. The collection of sufficient samples for ribociclib, vismodegib and vorinostat is still ongoing.

4. Conclusion

We successfully developed a sensitive LC–MS/MS assay for the simultaneous quantification of alectinib, cobimetinib, lenvatinib, nintedanib, osimertinib, palbociclib, ribociclib, vismodegib and vorinostat in human plasma. The validated linear assay ranges are 10–200 ng/mL for alectinib, lenvatinib, nintedanib and vismodegib, 50–1000 ng/mL for cobimetinib and palbociclib, 25–500 ng/mL for vorinostat, 100–2000 for osimetinib and 5–100 for ribociclib. Stability showed that, with the exception of osimertinib, all analytes were stable in human K_2EDTA plasma at room temperature for longer than 4 h. This assay is considered suitable to facilitate TDM.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2019.06.034.

References

[1] B. Gao, S. Yeap, A. Clements, B. Balakrishnar, M. Wong, H. Gurney, Evidence for therapeutic drug monitoring of targeted anticancer therapies, J. Clin. Oncol. 30 (2012) 4017–4025, http://dx.doi.org/10.1200/JCO.2012.43.5362.
[2] R.B. Verheijen, J.H. Beijnen, J.H.M. Schellens, A.D.R. Huitema, N. Steeghs, Clinical pharmacokinetics and pharmacodynamics of pazopanib: towards optimized dosing, Clin. Pharmacokinet. (2017) 1–11, http://dx.doi.org/10.1007/s40262-017-0510-z.
[3] K. Heinig, K. Miya, T. Kamei, E. Guerini, D. Fraier, L. Yu, S. Bansal, P.N. Morcos, Bioanalysis of alectinib and metabolite M4 in human plasma, cross-validation and impact on PK assessment, Bioanalysis 8 (2016) 1465–1479.
[4] E. Cardoso, T. Mercier, A. Dorothea, K. Homicsko, O. Michielin, K. Ellefsen-lavoie, L. Cagnon, M. Diezi, T. Buclin, N. Widmer, C. Csajka, L.

Decosterd, Quantification of the next-generation oral anti-tumor drugs dabrafenib, trametinib, vemurafenib, cobimetinib, pazopanib, regorafenib and two metabolites in human plasma by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B 1083 (2018) 124–136, http://dx.doi.org/10.1016/j.jchromb.2018.02.008.

[5] A.C. Dubbelman, H. Rosing, B. Thijssen, A. Gebretensae, L. Lucas, H. Chen, R. Shumaker, J.H.M. Schellens, J.H. Beijnen, Development and validation of LC – MS / MS assays for the quantification of E7080 and metabolites in various human biological matrices, J. Chromatogr. B 887–888 (2012) 25–34, http://dx.doi.org/10.1016/j.jchromb.2012.01.004.

[6] R. Reis, L. Labat, M. Allard, P. Boudou-rouquette, J. Chapron, A. Bellesoeur, A. Thomas-schoemann, J. Arrondeau, F. Giraud, J. Alexandre, M. Vidal, Journal of Pharmaceutical and Biomedical Analysis Liquid chromatography-tandem mass spectrometric assay for therapeutic drug monitoring of the EGFR inhibitors afatinib, erlotinib and osimertinib, the ALK inhibitor crizotinib and the VEGFR inhibitor nint, J. Pharm. Biomed. Anal. 158 (2018) 174–183, http://dx.doi.org/10.1016/j.jpba.2018.05.052.

[7] Y. Deng, H. Wong, R.A. Graham, W. Liu, H. Shen, Y. Shi, L. Wang, M. Meng, V. Malhi, X. Ding, B. Dean, Determination of unbound vismodegib (GDC-0449) concentration in human plasma using rapid equilibrium dialysis followed by solid phase extraction and high-performance liquid chromatography coupled to mass spectrometry, J. Chromatogr. B 879 (2011) 2119–2126, http://dx.doi.org/10.1016/j.jchromb.2011.05.048.

[8] P. Suresh, V. Devaraj, N. Srinivas, R. Mullangi, Review of bioanalytical assays for the quantitation of various HDAC inhibitors such as vorinostat, belinostat, panobinostat, romidepsin and chidamine, Biomed. Chromatogr. 31 (2017).

[9] M. Herbrink, N. de Vries, H. Rosing, A.D.R. Huitema, B. Nuijen, J.H.M. Schellens, J.H. Beijnen, Development and validation of a liquid chromatography-tandem mass spectrometry analytical method for the therapeutic drug monitoring of eight novel anticancer drugs, Biomed. Chromatogr. (2017), e4147, http://dx.doi.org/10.1002/bmc.4147.

[10] US Food Drug Administration (FDA), FDA Guidance for Industry: Bioanalytical Method Validation (2001), n.d.

[11] European Medicines Agency (EMA), Guideline on Bioanalytical Method Validation. Committee for Medicinal Products for Human Use and European Medicines Agency, 2011, London, (n.d.).

[12] P.N. Morcos, L. Yu, K. Bogman, M. Sato, H. Katsuki, K. Kawashima, D.J. Moore, M. Whayman, K. Nieforth, K. Heinig, E. Guerini, D. Muri, M. Martin-Facklam, A. Phipps, Absorption, distribution, metabolism and excretion (ADME) of the ALK inhibitor alectinib: results from an absolute bioavailability and mass balance study in healthy subjects, Xenobiotica 47 (2017) 217–229, http://dx.doi.org/10.1080/00498254.2016.1179821.

[13] G.K. Abou-Alfa, L.D. Lewis, P. LoRusso, M. Maitland, P. Chandra, S. Cheeti, D. Colburn, S. Williams, B. Simmons, R.A. Graham, Pharmacokinetics and safety of vismodegib in patients with advanced solid malignancies and hepatic impairment, Cancer Chemother. Pharmacol. 80 (2017) 29–36, http://dx.doi.org/10.1007/s00280-017-3315-8.

[14] J.J.M. Rood, M.T.J. Van Bussel, J.H.M. Schellens, J.H. Beijnen, R.W. Sparidans, Liquid chromatography – tandem mass spectrometric assay for the T790M mutant EGFR inhibitor osimertinib (AZD9291) in human plasma, J. Chromatogr. B 1031 (2016) 80–85, http://dx.doi.org/10.1016/j.jchromb.2016.07.037.