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Liquid chromatography-tandem mass spectrometry assay for the quantification of niraparib and its metabolite M1 in human plasma and urine

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ABSTRACT

Niraparib (MK-4827) is a novel poly(ADP-Ribose) polymerase (PARP) inhibitor currently investigated in phase III clinical trials to treat cancers. The development of a new drug includes the characterisation of absorption, metabolism and excretion (AME) of the compound. AME studies are a requirement of regulatory agencies and for this purpose bioanalytical assays are essential. This article describes the development and validation of a bioanalytical assay for niraparib and its carboxylic acid metabolite M1 in human plasma and urine using liquid chromatography-tandem mass spectrometry (LC–MS/MS). Sample pre-treatment involved protein precipitation for plasma and dilution of urine samples using acetonitrile-methanol (50:50, v/v). Final extracts were injected onto a SunFire C18 column and gradient elution using 20 mM ammonium acetate (mobile phase A) and formic acid:acetonitrile:methanol (0.1:50:50, v/v/v) (mobile phase B) was applied. Detection was performed on an API5500 tandem mass spectrometer operating in the positive electrospray ionisation mode applying multiple reaction monitoring (MRM). The assay was successfully validated in accordance with the Food and Drug Administration and latest European Medicines Agency guidelines on bioanalytical method validation and can therefore be applied in pharmacological clinical studies.

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1. Introduction

Niraparib (MK-4827) is a novel poly(ADP-Ribose) polymerase (PARP) inhibitor currently evaluated in cancer patients in phase III clinical trials at a dose level of 300 mg orally once daily [1,2]. Its mechanism of action has not been completely elucidated, but it is proposed that it lies in its ability to inhibit pathways involved in DNA repair processes [3]. Normal cellular functions, as well as everyday environmental stresses can lead to breaks in DNA. The principal DNA breaks are the single strand breaks (SSB), which are repaired through the base excision repair (BER) pathway, where the enzymes PARP-1 and PARP-2 play a vital role [4,5]. If these SSBs go unrepaired, they can result, especially during replication, in the more serious double strand DNA breaks (DSB). These DSBs are repaired through other mechanisms, including the homologous recombination repair (HRR) pathway and the non-homologous end joining (NHEJ) [3]. It is understood that by targeting cells that have a mutation in DSB repair mechanisms, the addition of a PARP inhibitor such as niraparib can cause apoptosis. This concept is referred to as synthetic lethality: where two individual mutations on their own are not lethal, the combination of these mutations can lead to cell death [6]. In the current context, synthetic lethality

Abbreviations: ACN, acetonitrile; BER, base excision repair; C.V., coefficient of variation; DMSO, dimethylsulfoxide; DSB, double strand break; EMA, European Medicine Agency; FDA, Food and Drug Administration; GLP, Good Laboratory Practice; HRR, homologous recombination repair; IS, internal standard; LC–MS/MS, liquid chromatography – tandem mass spectrometry; LLOQ, lower limit of quantification; MeOH, methanol; MF, matrix factor; MRM, multiple reaction monitoring; NHEJ, non-homologous end joining; OECD, Organisation for Economic Co-operation and Development; PARP, poly(ADP-ribose) polymerase; SSB, single strand break; ULOQ, upper limit of quantification.

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refers to the impairment of DNA repair due to PARP inhibition with genetically predisposed DNA repair deficiencies, specifically the BRCA-1 and BRCA-2 mutations. At the same time, PARP inhibitors can sensitise cancerous cells to other therapies that are targeted at inhibiting DSB repair [3].

Similar to other compounds in the process of registration, it is required to conduct a mass balance study to elucidate the disposition and elimination of a compound. A mass balance study requires an appropriate bioanalytical assay to allow the quantification of the parent compound and known metabolites. Moreover, such an assay can be used to quantify the parent compound in an absolute bioavailability study. To the best of our knowledge, no such method has been described before. This article describes the validation of a bioanalytical method for both niraparib and its known carboxylic acid metabolite M1 in plasma and urine for the support of clinical studies such as the mass balance study and the absolute bioavailability study mentioned above. This method was validated in compliance with the Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) [7] and in accordance to the Food and Drug Administration (FDA) and latest European Medicines Agency (EMA) guidelines on bioanalytical method validation [8,9].

2. Experimental

2.1. Chemicals

Niraparib reference standard (Fig. 1) was supplied by Dishman (Dist. Ahmedabad, India), and its deuterated internal standard (IS) M002151 was manufactured by Merck (Kenilworth, NJ, USA). M1 reference standard (Fig. 1) was supplied by Metrics Inc (Greenville, NC, USA), and its deuterated internal standard D5-M1 was provided by GLSynthesis Inc. (Worcester, MA, USA). Acetonitrile (ACN), methanol (MeOH) and water (all Supra-Gradient grade) were purchased from Biosolve Ltd (Valkenswaard, The Netherlands). ≥98% formic acid (analytical grade) and dimethylsulfoxide (DMSO) were supplied by Merck (Amsterdam, the Netherlands). Ammonium acetate (LC–MS grade) was purchased from Sigma Aldrich (Zwijndrecht, the Netherlands). Blank human dipotassium ethylenediaminetetraacetic acid (K_2EDTA) plasma was obtained from the department of clinical chemistry (MC Slotervaart, the Netherlands) and blank urine was obtained from healthy volunteers.

2.2. Stock solutions, calibration standards and quality control samples

Stock solutions from two separate weighings of niraparib, M1 and respective internal standards M002151 and D5-M1 were prepared by dissolving the analytes in dimethylsulfoxide, giving a final concentration of 0.1 mg/mL for both niraparib and M1, and a concentration of 1 mg/mL for both IS. Working solutions for calibration standards (CS) and quality control (QC) samples were obtained using different preparations of stock solutions by diluting niraparib together with M1 in water-ACN (50:50, v/v).

The internal standard working solution was obtained by diluting the IS stock solutions 200 times in 20 mM ammonium acetate in water-ACN (80:20, v/v), yielding a solution of 500 ng/mL. All working solutions were stored at −20 °C.

For each validation run, fresh CS were prepared by spiking 5 μL of the working solution to 95 μL blank matrix to obtain concentrations of 1, 2, 10, 50, 100, 250, 400 and 500 ng/mL for niraparib and M1 in plasma, and 1, 2, 5, 10, 25, 50, 80, 100 ng/mL in urine. QC samples were prepared in larger quantities by adding 125 μL of the working solutions to 2375 μL blank matrix to obtain concentrations of 1 (QC LLOQ), 3 (QC Low), 50 (QC Mid) and 375 (QC High) ng/mL for niraparib and M1 in plasma, and concentrations of 1, 3, 25 and 75 ng/mL for urine. The QC samples were stored at −70 °C in aliquots of 100 μL in 1.5 mL eppendorf tubes for the duration of the validation.

Additional working solutions were prepared for stability testing, as stability was to be assessed for niraparib and M1 separately. These stability-working solutions were spiked in plasma and urine at QC Low and QC High levels and stored at −20 °C.

2.3. Sample preparation

A volume of 25 μL of IS working solution of 500 ng/mL was added to a volume of 100 μL of biomatrix yielding a final IS concentration of 100 ng/mL. The samples were mixed and 300 μL ACN-MeOH (50:50, v/v) was added to precipitate the plasma proteins and to dilute urine. Samples were mixed again and centrifuged for 5 min at 15,000 rpm. The supernatant was transferred to clean eppendorf tubes before evaporating to dryness (at 40 °C) under a gentle stream of nitrogen. Dry extracts were reconstituted using 200 μL of the 20 mM ammonium acetate–ACN solution (80:20, v/v). Samples were centrifuged again at 15,000 rpm for 5 min and transferred to autosampler vials with inserts before analysis. A volume of 3 μL of the final extract was injected into the chromatographic system.

2.4. Instrumentation and operating conditions

2.4.1. Liquid chromatography

Chromatographic separation of niraparib and M1 was carried out using a high performance liquid chromatography (HPLC) Acquity I Class pump (Waters, Milford, MA, USA). Analyses were performed using a SunFire C18 column (50 mm × 2.1 mm, 5 μm) and samples were injected using a Class I HPLC autosampler (Waters) (thermostated at 8 °C). Analytes were separated using gradient elution with 20 mM ammonium acetate in water (mobile phase A) and 0.1% formic acid in ACN-MeOH (50:50, v/v). A flow rate of 700 μL per minute was applied through the column and the column oven was set to 40 °C. The elution gradient was set in such a way that it combined step-wise and ramp-wise changes: mobile phase B: 20% (initial), from 20 to 65% (0.02–0.52 min), 65% (0.52–2 min) from 65 to 90% (2–2.01 min), 90% (2.01–3.53 min), to 20% (3.53–3.54 min), 20% (3.54–4 min), from 20 to 100% (4–4.01 min), 100% (4.01–5.01 min), from 100 to 20% (5.01–5.02 min), 20% (5.02–5.52 min), from 20 to 100% (5.52–5.53 min), 100% (5.53–6.53 min), to 20% (6.53–6.54 min), 20% (6.54–7 min). The step-wise changes between 4 min and 6.54 min was used to eliminate carry-over, whereas the final 0.46 min was applied for column equilibration to initial conditions. The divert valve was set in place to direct the flow to the mass spectrometer from 0.8 to 3.0 min and to the waste for the remainder of the acquisition time to protect the mass spectrometer from contaminants.

2.4.2. Mass spectrometry

An API5500 tandem mass spectrometer (Sciex, Framingham, MA, USA) was used. Data acquisition was performed using Analyst 1.5.2 software (Sciex). Analyses were performed in the positive ion mode by multiple reaction monitoring (MRM), selecting precursor ions m/z 321 for niraparib, m/z 322 for M1, m/z 328 for M002151 and m/z 329 for D5-M1. Product ions of m/z 304 were selected for all analytes and IS (Fig. 2). Collision gas was set at 7 arbitrary units (a.u.) and curtain gas (nitrogen) flow was set at 40 a.u. The instrument was operated in positive electrospray ionisation (+ESI) mode. The source temperature was set to 650 °C and the ion spray voltage at +4500. The dwell time was 15 msec for both niraparib and M1. The declustering potential was set at 166 V for niraparib and

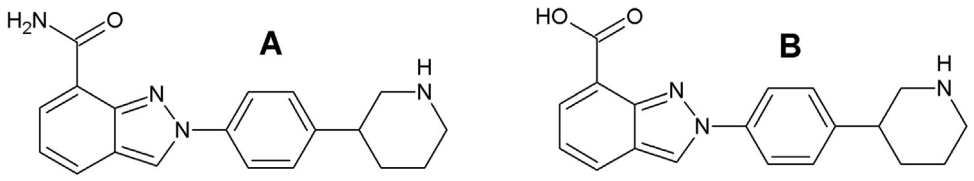

Fig. 1. Chemical structure of niraparib (A) and M1 (B).

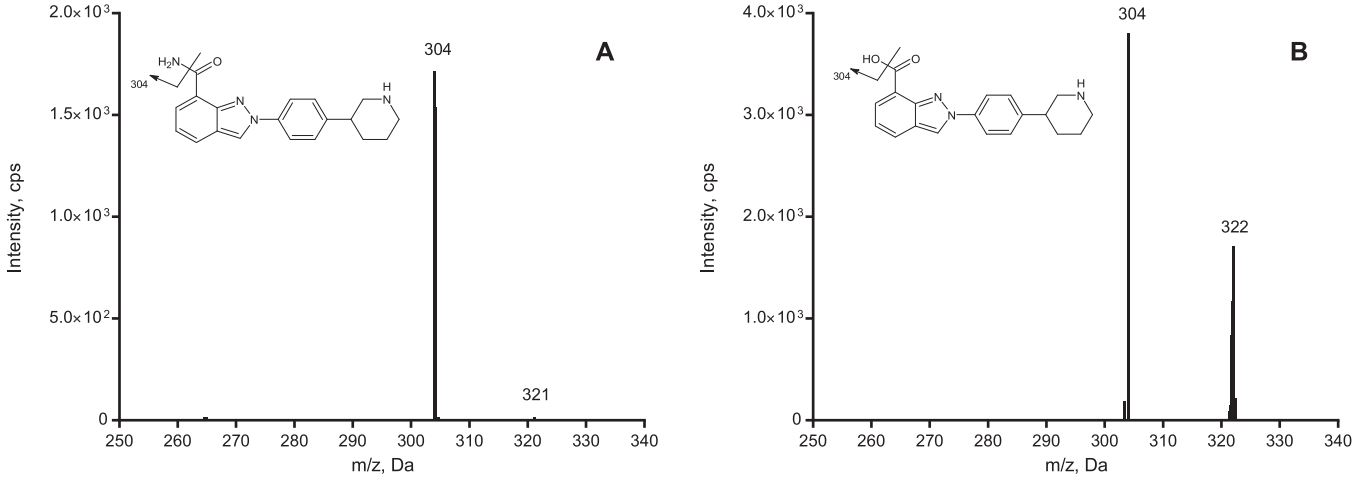

Fig. 2. Product ion spectra of $[M+H]^+$ ions of niraparib (A) and M1 (B).

M002151 and at 171 V for M1 and D5-M1. The entrance potential was 10 V for all analytes.

2.5. Validation procedures

A complete validation of the bioanalytical assay for plasma and urine was performed according to the regulatory guidelines [8,9], and included calibration curve, accuracy and precision, carry-over, selectivity, dilution integrity, matrix effect, recovery and stability. The validation was performed on the instruments with the settings as mentioned in Sections 2.4.1 and 2.4.2.

3. Results and discussion

3.1. Method development

3.1.1. Chromatography

The difference in mass between niraparib and M1 is 1 amu. The development of the chromatography mainly focused on the chromatographic separation of the two analytes. Another difficulty was the appearance of the C_{13}-niraparib peak in the transition window of M1. It was therefore essential to obtain chromatographic separation to ensure the correct quantification of M1. Experiments were carried out using different compositions of mobile phases. Experiments showed that a mobile phase of methanol alone did not result in chromatographic separation. However, a mobile phase consisting of acetonitrile only produced asymmetrical peaks. The combination of the two with the addition of 0.1% formic acid resulted in well separated peaks, with M1 being eluted out earlier, and reduced the tailing, thus optimising peak shape. Figs. 3 and 4 show representative LC–MS/MS chromatograms for niraparib (t_R: 1.26 min), M1 (t_R: 0.93 min) and respective internal standards in plasma and urine, respectively.

3.2. Method validation

3.2.1. Calibration curve

Calibration standards (8 non-zero) with a concentration range of 1–500 ng/mL for plasma and 1–100 ng/mL for urine were prepared in blank human K_2EDTA plasma and blank human urine, respectively, and analysed in duplicate in 3 separate analytical runs. Linear regression with a weighting factor of $1/x^2$ was applied, where x equals the concentration of niraparib or M1. The calibration curves were acceptable if at least 75% of all non-zero CS were within ±15% of the nominal concentrations, or ±20% for the lower limit of quantification (LLOQ). These acceptance criteria were met and thus the calibration curves were accepted for both plasma and urine.

3.2.2. Accuracy and precision

Five replicates of QC LLOQ (1 ng/mL), QC Low (3 ng/mL), QC Mid (50 ng/mL) and QC High (375 ng/mL) for plasma method validation were analysed in 3 analytical runs. Five replicates of QC LLOQ (1 ng/mL), QC Low (3 ng/mL), QC Mid (25 ng/mL) and QC High (75 ng/mL) for urine method validation were analysed in 3 analytical runs as well.

To assess accuracy and precision, expressed as the bias and the coefficient of variation, respectively, the following equations were used:

$$Intra-run\ bias\,(\%) = 100\% \cdot \frac{(mean\ measured\ conc.per\ run - nominal\ conc.)}{(nominal\ conc.)} \quad (1)$$

$$Overall\ bias\,(\%) = 100\% \cdot \frac{(overall\ mean\ measured\ conc. - nominal\ conc.)}{(nominal\ conc.)} \quad (2)$$

$$Intra-run\ CV\,(\%) = 100\% \cdot \frac{(SD\ of\ the\ measured\ conc.per\ run)}{(mean\ measured\ conc.per\ run)} \quad (3)$$

$$Inter-run\ CV\,(\%) = 100\% \cdot \frac{(SD\ of\ the\ overall\ measured\ conc.)}{(overall\ ,\ mean\ measured\ conc.)} \quad (4)$$

The acceptance criteria were met if two-third of the accuracy and precision calculations were within ±20% for the LLOQ level and within 15% at the other QC levels. Table 1 shows that all acceptance criteria were met.

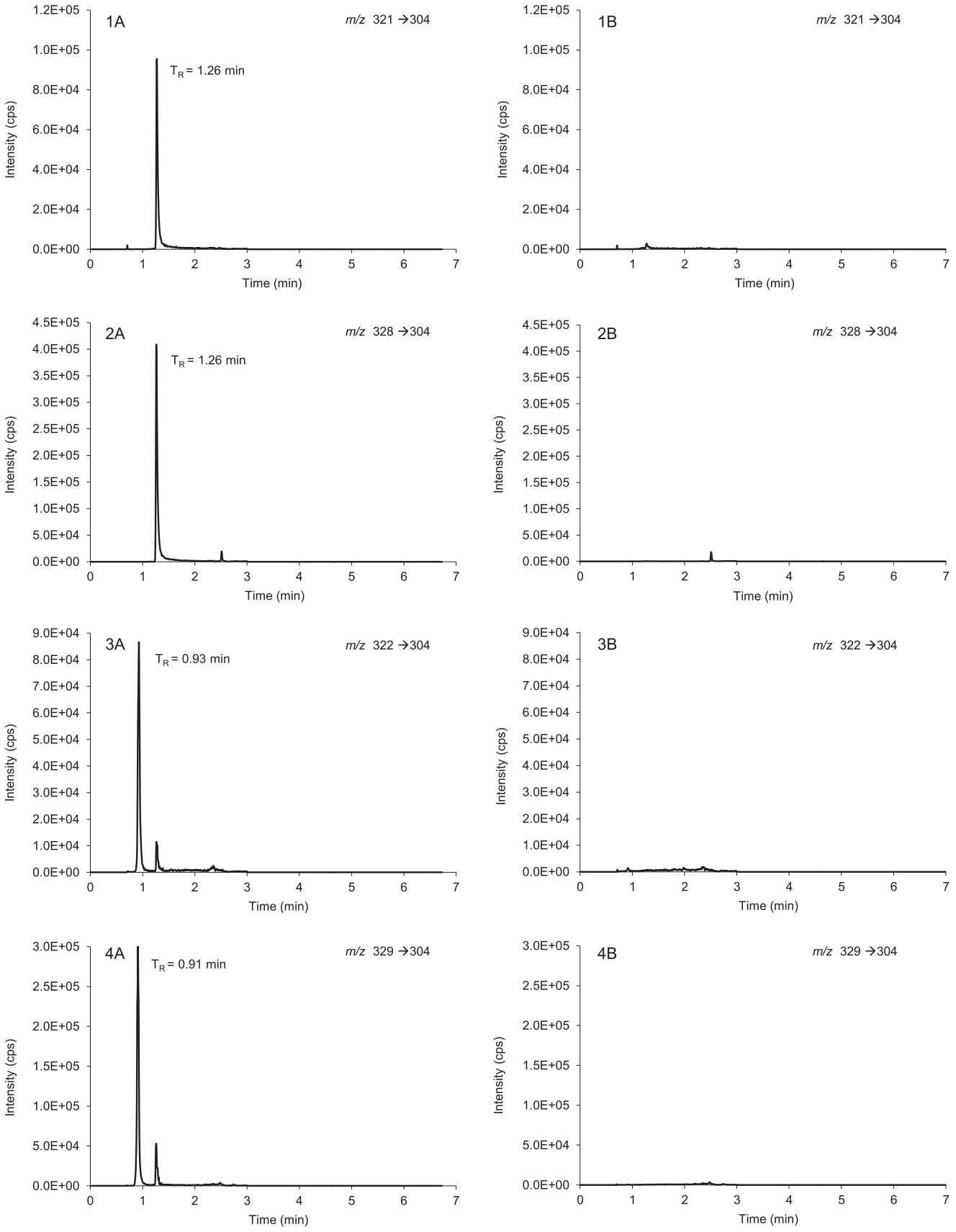

Fig. 3. MRM chromatograms of niraparib (1A), niraparib-IS (2A), M1 (3A) and M1-IS (4A) in plasma at LLOQ level (1 ng/mL for niraparib and M1, 500 ng/mL for the internal standards) and chromatograms in a blank sample of niraparib (1B), niraparib-IS (2B), M1 (3B) and M1-IS (4B).

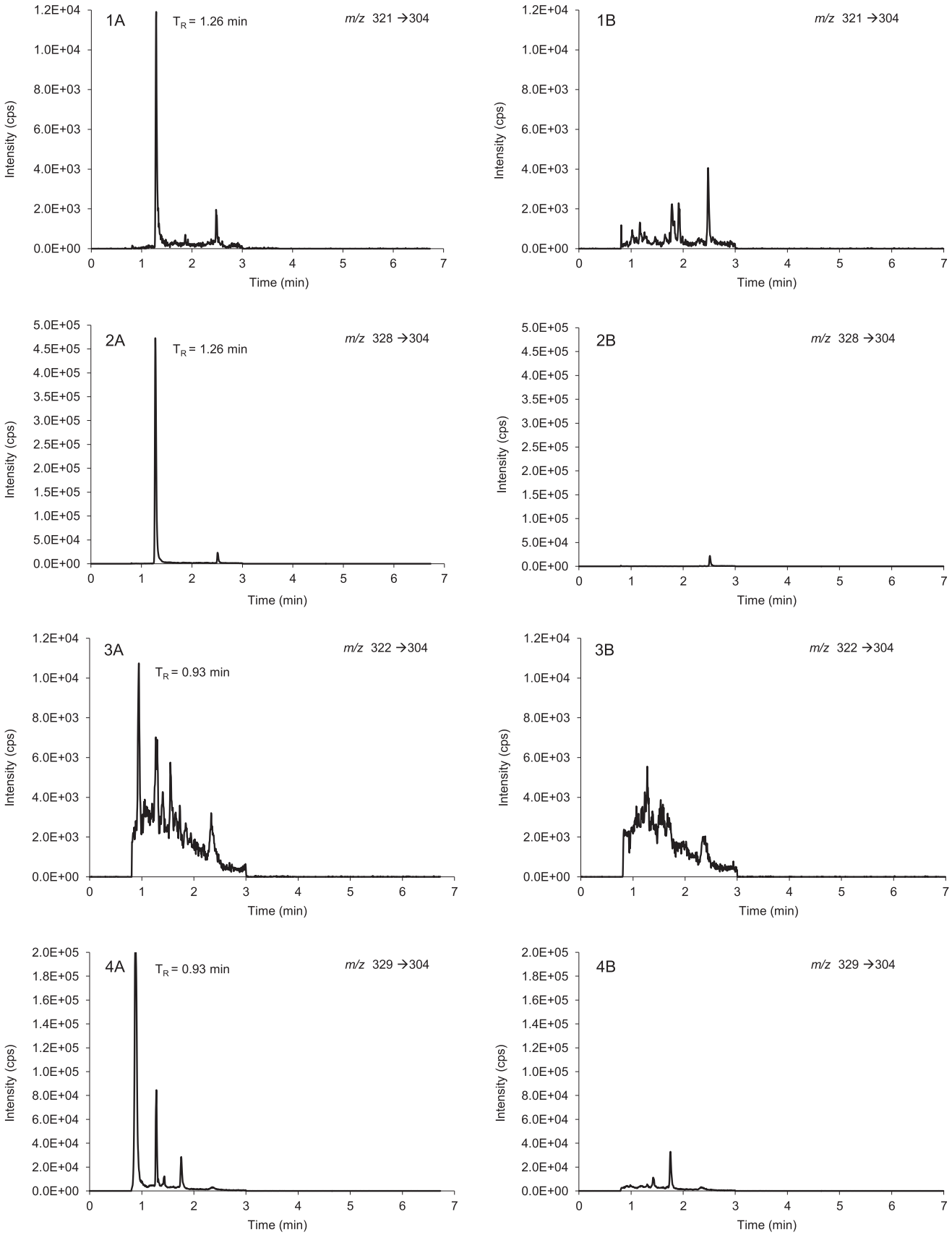

Fig. 4. MRM chromatograms of niraparib (1A), niraparib-IS (2A), M1 (3A) and M1-IS (4A) in urine at LLOQ level (1 ng/mL for niraparib and M1, 500 ng/mL for the internal standards) and chromatograms in a blank sample of niraparib (1B), niraparib-IS (2B), M1 (3B) and M1-IS (4B).

Table 1
Assay performance data for the analysis of niraparib and M1 in human plasma and urine.

Matrix	Analyte	Nom. conc. (ng/mL)	n	Intra-assay		Inter-assay	
				Bias[a] (%)	CV[a] (%)	Bias (%)	CV (%)
Plasma	Niraparib	1.00	15	−2.1 – 5.5	3.7–10.7	1.9	0.7
		3.00	15	−3.8 – −0.2	2.2–4.7	−2.2	0.9
		50.0	15	1.6–2.3	1.2–2.2	1.9	−*
		375	15	2.0–3.3	4.1–5.1	2.9	−*
	M1	1.00	15	0.1–9.2	3.3–7.0	3.9	4.0
		3.00	15	−2.3 – 1.9	1.8–5.6	0.3	1.4
		50.0	15	−2.0 – 5.7	1.7–3.5	3.0	4.0
		375	15	0.4–3.6	4.6–5.9	1.7	−*
Urine	Niraparib	1.00	15	0.5–8.5	2.6–7.6	4.7	3.1
		3.00	15	−0.9 – 4.7	1.7–3.9	2.7	2.7
		50.0	15	1.5–5.0	2.0–3.9	3.0	1.2
		375	15	0.6–4.0	1.1–3.4	2.7	1.5
	M1	1.00	15	1.5–12.2	3.9–12.7	7.7	3.5
		3.00	15	6.3–12.1	2.1–3.8	8.8	2.4
		50.0	15	5.0–9.7	2.1–4.4	8.0	2.0
		375	15	6.3–12.3	1.6–3.7	9.8	2.5

Nom.: nominal; conc.: concentration; n: number of replicates; CV: coefficient of variation; [a] If multiple validation runs were performed, the range of accuracies and precisions was listed; *: Inter-run precision could not be calculated (mean square between group is less then mean square within groups).

Table 2
Stability data for niraparib and M1 in plasma and urine. All experiments in the biomatrix were performed in triplicate at low and high concentrations (3 ng/mL and 375 ng/mL in plasma, 3 ng/mL and 75 or 750 ng/mL[a] in urine).

Conditions	Matrix	Analyte	Nom. conc. (ng/mL)	Measured conc. (ng/mL)	Bias (%)	CV (%)	n
Plasma							
5 freeze-thaw cycles (−70 °C/ambient)	Biomatrix	Niraparib	3.00	2.78	−7.3	6.5	3
			375	378	0.8	4.7	3
		M1	3.00	3.14	4.6	5.0	3
			375	398	6.1	1.1	3
Ambient, 4 h	Biomatrix	Niraparib	3.00	2.67	−11.1	2.4	3
			375	375	0.1	5.4	3
		M1	3.00	2.84	−5.4	6.7	3
			375	364	−3.0	3.5	3
2–8 °C, 6 days	Final extract	Niraparib	3.00	2.97	−1.0	5.5	3
			375	368	−1.9	1.1	3
		M1	3.00	3.36	12.1	18.3	3
			375	386	2.9	2.3	3
2–8 °C, 3 days	Final extract (re-injection)	Niraparib	3.00	3.06	1.9	1.1	3
			375	383	2.2	1.4	3
		M1	3.00	3.03	1.1	2.9	3
			375	389	3.6	2.1	3
Whole blood							
Ice-water, 2 h	Biomatrix	Niraparib	375	429	9.4	3.5	3
		M1	375	553	−11.3	5.7	3
Urine							
5 freeze-thaw cycles (−70 °C/ambient)	Biomatrix	Niraparib	3.00	3.10	3.4	2.4	3
			750.0	702	−6.4	4.6	3
		M1	3.00	3.04	1.3	4.9	3
			750.0	731	−2.5	1.5	3
Ambient, 4 h	Biomatrix	Niraparib	3.00	2.87	−4.4	3.3	3
			750.0	730	−2.7	1.7	3
		M1	3.00	2.86	−4.6	3.8	3
			750.0	776	3.5	10.9	3
2–8 °C, 24 h	Biomatrix	Niraparib	3.00	3.12	4.1	4.3	3
			750.0	707	−5.7	1.7	3
		M1	3.00	2.86	−4.7	0.7	3
			750.0	757	0.9	1.7	3
2–8 °C, 21 days	Final extract	Niraparib	3.00	3.09	3.0	2.3	3
			750.0	692	−7.7	0.8	3
		M1	3.00	2.98	−0.8	4.2	3
			750.0	737	−1.8	2.7	3
2–8 °C, 4 days	Final extract (re-injection)	Niraparib	3.00	3.06	1.9	1.1	3
			75.0	76.1	1.3	2.8	3
		M1	3.00	3.29	9.7	1.1	3
			75.0	81.6	8.8	3.4	3

Nom.: nominal; conc.: concentration; n: number of replicates; CV: coefficient of variation. [a] 750 ng/mL was tested to cover the expected concentration range in patient urine samples. These samples were diluted ten times.

3.2.3. Specificity and selectivity

Six different batches of blank human K_2EDTA plasma and urine were spiked at the LLOQ level and were processed together with double blank samples to assess the selectivity of the assay. LC–MS/MS chromatograms of the blanks and LLOQ samples were monitored and compared for chromatographic integrity and potential interferences. The maximum interference from niraparib in the double blank samples was 9.2% of the LLOQ peak area in plasma and 18.1% of the LLOQ peak area in urine, while no interference was observed from M1 in both matrices, nor from both IS. The tests for endogenous interferences were considered acceptable, since no interferences from endogenous material at the retention time of the analyte with areas >20% (or >5% for the internal standards) of the LLOQ areas were observed in the blanks.

Cross-analyte interferences were tested by spiking blank human plasma and blank human urine with niraparib, M1, M002151 and D5-M1 separately at its upper limit of quantification (ULOQ). For each spiked sample, the interferences at the retention times and mass transitions of the analyte and IS were evaluated. The interference of the IS in the niraparib transition was found to be 11.9% in plasma and 3.4% in urine. The maximum interference observed from the M1-IS on M1 was found to be 9.2%. A small interference of 0.9% was observed from niraparib-IS in the M1-IS transition. In urine, no interference from either analytes was found. For all analyte/IS, retention time/mass transition combinations, the interference was <20% (or 5% for IS) of the LLOQ peak area and therefore considered acceptable.

3.2.4. Dilution integrity

Dilution integrity was assessed for niraparib and M1 in plasma as well as urine. Five replicates were spiked at a concentration above the ULOQ; 2000 ng/mL and 750 ng/mL for plasma and urine, respectively, which were diluted a ten-fold. All results fell within the acceptance criteria of ±15% and ≤15% for accuracy and precision, respectively. From these data it can be concluded that samples exceeding the ULOQ can be diluted using a dilution factor of ≤10.

3.2.5. Lower limit of quantification

The signal-to-noise ratio at the LLOQ was at least 23 and 6 for niraparib in plasma and urine, respectively, and at least 12 and 8 for M1 in plasma and urine, respectively. These values were obtained by comparing the signal at the LLOQ to the response in a blank sample. The analyte response at the LLOQ was at least 5 times the blank sample response and therefore considered acceptable.

3.2.6. Matrix effect

Niraparib and M1 were spiked to six different batches of blank human K_2EDTA and six different batches of urine at QC Low and QC High concentrations and compared to respective analyte in neat solution. The matrix factor (MF) was calculated for each batch of matrix as the ratio of the peak area in the presence of matrix to the peak area in absence of matrix. Furthermore, the IS-normalised MF was calculated using the following formula:

$$\textit{IS-normalised MF} = \frac{\textit{MF of the analyte}}{\textit{MF of the internal standard}}.$$

For niraparib, the coefficient of variation of IS-normalised MF from the 6 batches appeared to be below 2.4% for plasma, and below 5.5% for urine. For M1 this was 4.8% and 6.0% for plasma and urine, respectively. At both tested concentration levels in both matrices the coefficients of variation of the IS-normalised MF from the 6 batches were <15% for niraparib and for M1, thus meeting the acceptance criteria.

The effect of haemolysis was tested during plasma validation. Here, niraparib and M1 were spiked to 5% haemolysed plasma (which is whole blood kept at −20 °C for at least 12 h diluted with control plasma) at low and high concentrations (3 ng/mL and 375 ng/mL) in triplicate. For niraparib, accuracy and precision ranged from 2.7% to 4.7% and 6.4% to 13.1%, respectively, and accuracy and precision values for M1 ranged from 2.1% to 7.7% and from 4.8% to 12.8%, respectively. Values were compared to the nominal concentration and were found to fulfil the requirements.

3.2.7. Recovery

The sample pre-treatment recovery and total recovery were investigated in one batch of control human plasma and control human urine at low and high concentration (3 ng/mL and 375 ng/mL in plasma, 3 ng/mL and 75 ng/mL in urine) in triplicate. For the analytes and the internal standards, the sample pre-treatment recovery was calculated by dividing the peak area of the processed sample over the peak area in presence of matrix. The total recovery was similarly calculated by dividing the peak area in the processed sample over the peak area in absence of matrix. Both plasma and urine sample pre-treatment recovery for both the analyte and IS was around 80% and the total recovery around 75%.

3.2.8. Carry-over

Carry-over double blank samples injected after the ULOQ showed the presence of a peak at the retention time of niraparib in plasma, which was determined to be 144% of the area of the LLOQ. Carry-over was reduced to 18.8% in the third carry-over sample. Since this does not meet the acceptance criteria (≤20%) it was agreed that at least 3 double blank samples need to be injected after the ULOQ or after a sample which is suspected to have a high concentration of niraparib.

To minimise the carry-over effect, samples were arranged from lowest to highest niraparib concentrations. No carry-over problems were observed for M1 in plasma, and for both analytes in the urine matrix.

3.2.9. Stability

Stability was assessed for niraparib and M1 separately. For this reason additional working solutions were prepared for the two analytes at QC Low and QC High concentrations. Stock solution short term stability was tested at storage conditions of −20 °C and processing conditions (room temperature) for 45 days and 24 h, respectively. Before analysis, the stock solution (0.1 mg/mL) was diluted in triplicate to 100 ng/mL. Stability was assessed by comparing these solutions to freshly prepared stock solutions, diluted in the same manner. Acceptance criteria were met when the deviation from the fresh stock was within ±5%. As this was the case, it was concluded that stock solutions were stable for at least 24 h at room temperature and for at least 45 days at −20 °C.

All other stability experiments were performed in triplicate at QC High and QC Low concentrations. Measured concentrations were compared to those of freshly prepared QC samples and can be found in Table 2. Whereas stability tests in plasma were performed at QC Low and QC High concentrations (3 ng/mL and 375 ng/mL), stability in urine was assessed at QC Low and at a concentration above the ULOQ (750 ng/mL). The high concentrations were then diluted ten times, as it was observed that dilution integrity remained. This was done because niraparib and M1 were expected to be found in much higher concentrations, exceeding the calibration range, in patient urine samples.

4. Clinical application

Niraparib is a novel PARP inhibitor currently evaluated in clinical trials. The purpose of this method development and validation was to enable the support of pharmacological clinical studies, including a bioavailability study and a mass balance study where subjects

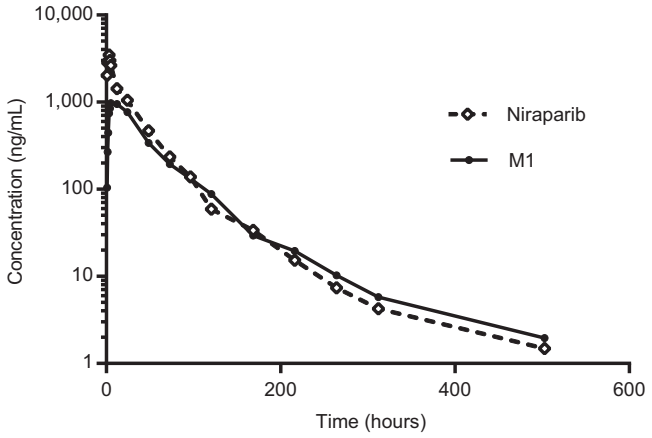

Fig. 5. Representative plasma concentration-time curves of niraparib and its metabolite M1 following an oral dose of 300 mg in a cancer patient.

received a single oral dose of 300 mg. In these studies blood samples were collected using K_2EDTA tubes and plasma was obtained by centrifugation. The validated bioanalytical method described in this article was applied to quantify niraparib and its main metabolite in these plasma samples. Fig. 5 shows an example of a plasma concentration-time curve resulting from PK analysis in one of these clinical studies The PK curves show that niraparib is rapidly absorbed, reaching a maximum concentration within 4 hours. Its main metabolite is formed quickly and shows a similar profile as niraparib.

5. Conclusion

Quantification methods for niraparib and its carboxylic acid M1 in plasma as well as in urine were validated according to the latest FDA and EMA guidelines [8,9]. This method validation includes all procedures required to show that the determination of niraparib concentrations in plasma and urine is reliable for the intended application. The quantifiable range for niraparib and M1 was 1–500 ng/mL for plasma and 1–100 ng/mL for urine. Samples with concentrations above the ULOQ can be reliably diluted 10 times using control matrix to fall within the validated calibration range. This assay has been used to support pharmacological clinical trials in which niraparib was administered orally to patients with advanced cancer.

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