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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.

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