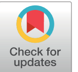
Journal of Chromatography B 1138 (2020) 121925

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Development and validation of an integrated LC-MS/MS assay for therapeutic drug monitoring of five PARP-inhibitors



M.A.C. Bruin[a,b,*], N. de Vries[a], L. Lucas[a], H. Rosing[a], A.D.R. Huitema[a,d], J.H. Beijnen[a,b,c]

[a] Department of Pharmacy & Pharmacology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[b] Division of Pharmacology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
[c] Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
[d] Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

ARTICLE INFO

Keywords:
LC-MS/MS
Validation
PARP-inhibitor
Therapeutic drug monitoring
Niraparib
Olaparib
Rucaparib
Talazoparib
Veliparib

ABSTRACT

An liquid chromatography-mass spectrometry (LC-MS/MS) assay was developed for the combined analysis of the five poly (ADP-ribose) polymerase (PARP) inhibitors niraparib, olaparib, rucaparib talazoparib and veliparib. A simple and fast sample pre-treatment method was used by protein precipitating of plasma samples with acetonitrile and dilution of the supernatant with formic acid (0.1% v/v in water). This was followed by chromatographic separation on a reversed-phase UPLC BEH C18 column and detection with a triple quadrupole mass spectrometer operating in the positive mode. A simplified validation procedure specifically designed for bioanalytical methods for clinical therapeutic drug monitoring (TDM) purposes, was applied. This included assessment of the calibration model, accuracy and precision, lower limit of quantification (LLOQ), specificity and selectivity, carry-over and stability. The validated range was 30–3000 ng/mL for niraparib, 100–10,000 ng/mL for olaparib, 50–5000 ng/mL for rucaparib, 0.5–50 ng/mL for talazoparib and 50–5000 for veliparib. All results were within the criteria of the US Food and Drug Administration (FDA) guidance and European Medicines Agency (EMA) guidelines on method validation. The assay has been successfully implemented in our laboratory.

1. Introduction

PARP-inhibitors are a relatively new class of targeted anti-cancer agents in the field of personalized medicine. Currently, the PARP-inhibitor veliparib is in a late stage of clinical development while niraparib, olaparib, rucaparib and talazoparib have recently been approved by the FDA and/or EMA. These four PARP-inhibitors are authorized as monotherapy for breast cancer gene (BRCA)-mutated or platinum-sensitive recurrent ovarian cancer and/or BRCA-mutated human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer [1,2]. Preclinical and clinical studies have shown promising results for PARP-inhibitors in more cancer types either as monotherapy or in combination with radiation, chemotherapeutics and other targeted agents [3–6]. Therefore, the field of PARP-inhibitor therapy is anticipated to expand rapidly.

Optimal clinical benefit from targeted anti-cancer agents relies highly on sufficient drug exposure. Drug exposure can be influenced by different factors such as individual pharmacokinetic variability in absorption, distribution and metabolism, the pharmacogenetic background of a patient, adherence to treatment and drug-drug interactions [6,7]. PARP-inhibitors are, like most targeted anti-cancer agents, administrated orally, given in a fixed dose and substrates to different metabolizing enzymes and transporters [8–12]. Consequently, large variability in drug levels and exposure of targeted anti-cancer agents between patients are frequently observed. Low drug levels may lead to suboptimal effects whereas high drug levels may cause side-effects. Poor tolerability of treatment and therapeutic failure can be the result, which might be prevented by treatment individualization [13].

A useful tool for treatment individualization is TDM. The basis for TDM is a clear relationship between exposure-response and/or exposure-toxicity. For a large number of therapeutic agents, a clear relationship between exposure and the efficacy of therapy has been described [14]. According to several clinical studies, such relationships might also exist for PARP-inhibitors [15–18]. This suggests the need for TDM of PARP-inhibitors and therefore the relationships between exposure-response and/or exposure-toxicity and optimal target drug concentrations should be further investigated.

An important condition for TDM is the availability of a reliable

* Corresponding author at: Department of Pharmacy & Pharmacology, Antoni van Leeuwenhoek – The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands.
E-mail address: ma.bruin@nki.nl (M.A.C. Bruin).

https://doi.org/10.1016/j.jchromb.2019.121925
Received 6 September 2019; Received in revised form 29 November 2019; Accepted 2 December 2019
Available online 06 December 2019
1570-0232/ © 2019 Elsevier B.V. All rights reserved.

assay for quantification of the therapeutic agent in e.g. plasma which allows for rapid sample turnover. Various LC-MS/MS assays have been reported for the quantification of the different PARP-inhibitors alone [19–25] or in combination with other agents [13,26–29], but not for the combined analysis of the five PARP-inhibitors for the specific purpose of TDM. Bioanalytical assays for TDM applications should be simple and fast, since these assays are widely used for routine clinical care. Recommendations from FDA and EMA guidelines on bioanalytical method validation are established for pharmacokinetic or toxicokinetic studies, however, they are not always applicable to TDM assays. Therefore we applied an adjusted validation protocol based on these guidelines, suitable for TDM assays [30]. Here we present an LC-MS/MS assay to simultaneously quantify the five PARP-inhibitors niraparib, olaparib, rucaparib, talazoparib and veliparib in human plasma for TDM purposes and its successful implementation in real life daily oncology practice.

2. Materials and methods

2.1. Chemicals

Niraparib, Olaparib, Rucaparib, Talazoparib, Veliparib, $^{13}C_6$-Niraparib as hydrochloride salt, $^{2}H_8$-Olaparib, $^{13}C,^{2}H_3$-Rucaparib, $^{13}C,^{2}H_4$-Talazoparib and $^{13}C,^{2}H_3,^{15}N$-Veliparib as dihydrochloride salt were purchased from Alsachim (Illkirch Graffenstaden, France). Formic acid 99%, methanol and water, used to prepare the mobile phase, together with acetonitrile, used for sample preparation, were obtained from Biosolve Ltd. (Valkenswaard, The Netherlands). Dimethylsulfoxide (DMSO), used to prepare stock solutions, was obtained from Merck (Darmstadt, Germany) and K_2EDTA blank human plasma were from BioIVT (Westbury, NY, USA).

2.2. Stock- and working solutions

Stock solutions containing niraparib, rucaparib, talazoparib and veliparib were prepared in DMSO and stored at −70 °C. Stock solutions of olaparib were prepared in DMSO-methanol (20:80, v/v) and stored at −20 °C. Internal standard (IS) stock solutions were prepared at the same concentration, in the same solvent, and stored at the same conditions as the corresponding analyte. In order to obtain calibration standards and quality control (QC) samples, working solutions were prepared in methanol-water (50:50, v/v) using separate stock solutions. Table S1 in the Supplementary material shows the prepared concentrations of the stock solutions and working solutions. An IS working solution was prepared in methanol-water (50:50, v/v) at concentrations of 1500 ng/mL for niraparib, 5000 ng/mL for olaparib, 50 ng/mL for talazoparib and 2500 ng/mL for rucaparib and veliparib by mixing IS stock solutions. The IS working solution was stored at −20 °C.

2.3. Calibration standards and quality control samples

Separately prepared working solutions were used to prepare the calibration standards and QC samples. A volume of 50 µL of working solution was spiked to 950 µL of human K_2EDTA plasma and subsequently aliquots of 50 µL were made and stored at −20 °C. The final concentrations are shown in Table S1 in the Supplementary material.

2.4. Sample preparation

Whole blood samples were collected from patients treated with niraparib, olaparib, rucaparib, talazoparib or veliparib. Directly after collection, samples were centrifuged for 10 min at 2000*g* at 4 °C. Thereafter, plasma was obtained and stored at −20 °C until analysis. Before sample pretreatment, samples were thawed at room temperature. To 50 µL of plasma, a volume of 10 µL IS working solution was added, except for the double blank samples. A volume of 100 µL acetonitrile was used for protein precipitation (PP) to extract the analytes from plasma. Samples were vortex-mixed for 5 s, shaken on an automatic shaker for 10 min at 1250 rpm and centrifuged at 23,100*g* for 5 min at room temperature. A volume of 75 µL supernatant was transferred to an autosampler vial with insert which contained 75 µL of 0.1% formic acid in water. The final extract was vortex-mixed and stored at 2–8 °C until analysis.

2.5. Analytical equipment and conditions

Chromatographic separation was achieved using a Nexera 2 series liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with a binary pump, a degasser, an autosampler, valco valve and column oven. The autosampler temperature was maintained at 4 °C and the column oven at 40 °C. The mobile phase consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in methanol (phase B). A block gradient (Table S2 in the Supplementary material) was used at a flowrate of 0.3 mL/min. A reversed phase Acquity UPLC BEH C18 column (100 × 2.1 mm, particle size 1.7 µm) was coupled to an Acquity UPLC BEH C18 Vanguard pre-column (5 × 2.1 mm, particle size 1.7 µm) (Waters, Milford, MA, USA) for protection. The flow was directed into the MS between 1.50 and 4.50 min and into the waste container during the remainder of the run using the divert valve. The chromatographic system was coupled to a triple quadrupole mass spectrometer 6500+ (Sciex, Framingham, MA, USA) equipped with a turbo ionspray interface (TIS) operating in the positive ion mode. By direct infusion of each analyte in 0.1% formic acid in 80% methanol, mass spectrometric parameters were optimized. The multiple reaction monitoring (MRM) mode was used with unique transitions for each analyte and IS. Data was acquired and processed using Analyst™ software version 1.6.2 (AB Sciex).

2.6. Validation procedure

Similar to a previously validated assay in our laboratory for TDM purposes [31], we followed a dedicated validation protocol suitable for TDM assays [30]. We used the fit-for purpose strategy according to van Nuland et al. [30] which is based on the FDA and EMA guidelines [32,33] and adjusted to TDM assays. We evaluated the calibration model, accuracy and precision, lower limit of quantification (LLOQ), specificity and selectivity, carry-over and stability. A reduced number of calibration standards were then used to increase the turnaround during the routine application of the method and QC samples were made at three levels (LLOQ, medium and upper limit of quantification (ULOQ)). To increase the robustness of the method a signal to noise (S/N) ratio of 10 was strived for. We use isotopically labeled IS's to correct for matrix effects and optimized recovery during the method development. Dilution integrity was not established, since the validated range will cover the majority of the concentrations in patient samples. The other mentioned validation parameters were evaluated according to the FDA and EMA guidelines.

2.7. Clinical application

The assay was developed and validated to support pharmacokinetic studies of TDM for the five PARP-inhibitors niraparib, olaparib, rucaparib, talazoparib and veliparib. After patients signed informed consent, K_2EDTA blood samples (4 mL) were collected from patients treated with one of the five PARP-inhibitors at the Antoni van Leeuwenhoek – The Netherlands Cancer Institute. Plasma samples were obtained as described in this article.

3. Results and discussion

3.1. Development

Calibration ranges were determined based on the recommended

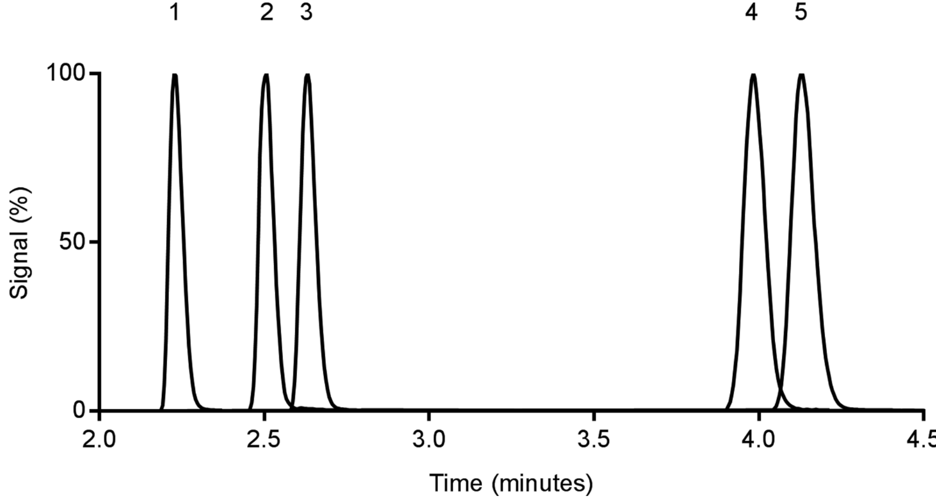


Fig. 1. Representative normalized LC-MS/MS chromatograms of spiked human plasma at QC medium concentrations: veliparib (1; 2500 ng/mL), rucaparib (2; 2500 ng/mL), niraparib (3; 1500 ng/mL), talazoparib (4; 25 ng/mL) and olaparib (5; 5000 ng/mL).

monotherapy dose by EMA [34–37] in combination with pharmacokinetic studies of the analytes [15–18,38–42]. In routine clinical care, blood withdrawal will not necessarily be performed at steady state. Therefore, calibration ranges were chosen based on average minimum observed plasma concentration (C_{min}) and average maximum observed plasma concentration (C_{max}) at steady state, to cover the majority of the concentrations in patient samples.

Previously developed bioanalytical methods for quantification of PARP-inhibitors used liquid-liquid extraction (LLE) or PP in combination with an evaporation step, which can be time consuming [19–22,26,29]. Since the assay will be used for routine care with a fast turnaround, a simple and fast sample preparation method was desirable. Therefore, PP was chosen for sample preparation, similar to some previous published methods [13,23,27,28,43]. Methanol, acetonitrile and methanol-acetonitrile (50:50, v/v) were evaluated as precipitation solvent. The three solvents resulted in good responses for all analytes, but acetonitrile showed the optimal response for talazoparib. To preserve sufficient sensitivity at LLOQ level for talazoparib and good efficiency in removing endogenous proteins, a ratio of 1:2 (biological sample:acetonitrile) was chosen for precipitation [44]. To correct for variability during sample pre-treatment, we used isotopically labelled internal standards, in contrast to previous methods developed for quantification of rucaparib and veliparib [20,23,26]. Direct injection of the supernatant onto the chromatographic system resulted in solvent effects for veliparib. The hydrophilic characteristics of veliparib and injection of a strong solvent onto the weak mobile phase can explain this effect. Therefore the supernatant was diluted (1:1) with 0.1% formic acid in water before injection. This resulted in an acceptable peak shape of veliparib and sufficient sensitivity at LLOQ level for talazoparib.

The combination of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in methanol:acetonitrile (50:50, v/v) (Eluent B) with a reversed phase Acquity UPLC BEH C18 column (100 × 2.1 mm, particle size 1.7 µm) resulted in symmetric peaks and the analytes responded well. Niraparib, rucaparib and veliparib were chromatographically separated, however, olaparib and talazoparib eluted at the same time from the column. Considering the use of isotopically labelled internal standards and the unique transitions for each analyte and IS, separation of the analytes is not required. However, when independently spiked talazoparib QC samples reflecting patient samples were quantified using calibration standards containing a mix of all analytes, the quantification of talazoparib was biased: a mean deviation of 10.8% was measured in talazoparib QC samples containing 50 ng/mL analytes. Talazoparib-IS is obviously not able to correct for the ion-suppression effects possibly caused by the high concentrations of olaparib and olaparib-IS. Therefore we changed eluent B into 0.1% formic acid in methanol to separate olaparib from talazoparib (Fig. 1) which improved the accuracy of the independently spiked talazoparib QC samples.

Optimal high sensitivity settings for niraparib, olaparib, rucaparib and veliparib resulted in a non-linear calibration model, due to saturation of the detector. A linear calibration model is desirable to preserve an adequate accuracy and precision around the upper limit of quantification (ULOQ). In this way, sensitivity is constant over the validated concentration range. Therefore, less sensitive product ions were chosen for niraparib, olaparib, rucaparib and veliparib. Additionally, collision energy was de-optimized for niraparib and veliparib, and the $[M+H]^+ + 1$ isotopologue – product transition was used for quantification of olaparib. General mass spectrometric settings were optimized for talazoparib, since the target LLOQ for this analyte represented the lowest concentration. However, the optimal source temperature of 750 °C for talazoparib resulted in in-source degradation of rucaparib. Therefore, the source temperature was maintained at 500 °C. A less sensitive product ion (*m/z* 298) was chosen for talazoparib as well, since the S/N ratio was better compared to the most sensitive product ion (*m/z* 285). A summary of the general and specific settings of the LC-MS/MS system are provided in Table S3 in the Supplementary material. Table 1 shows the structures of the five analytes, the formed product ions and the relative intensity of the product ions.

3.2. Validation procedures

Four calibration standards were analyzed in three analytical runs on three separate days to determine the linearity of the calibration model. We used linear regression of the analyte/IS peak area ratio vs concentration (x) with weighting factor $1/x^2$ to obtain the lowest absolute and total bias across the calibration ranges. The assay was linear for the concentration ranges of 30–3000 ng/mL for niraparib, 100–10,000 ng/mL for olaparib, 50–5000 ng/mL for rucaparib, 0.5–50 ng/mL for talazoparib and 50–5000 for veliparib. All calibration curves of the analytes (n = 3) were within the criteria and had bias within ± 15% (± 20% for LLOQ) of the nominal concentration for at least 75% of the calibration standards. The correlation coefficients were 0.993 or better.

Table 1
Molecular structure, used parent ion, generated product ions and the relative intensity of the product ions of niraparib, olaparib, rucaparib, talazoparib and veliparib.

Analyte	Parent ion (*m/z*)	Product ion (*m/z*)	Relative intensity (%)
Niraparib	321	304	100
		235	16
		205	7
Olaparib	436	367	100
		281	72
		253	7
		69	16
Rucaparib	324	293	100
		264	14
		236	28
Talazoparib	381	298	73
		285	100
		109	27
		84	23
Veliparib	245	162	100
		145	81
		117	23
		90	13
		84	97

Accuracy and precision were evaluated for each analyte by analyzing five replicates of the QC samples (LLOQ, medium and ULOQ) in three analytical runs on three separate days. The inter-assay accuracy, intra-assay accuracy and precision were calculated using the equations described by Herbrink et al. [45]. The highest value was observed for QC LLOQ of veliparib with an intra-assay C.V. of 7.8% (Table 2). All biases and C.V.s were below this value and therefore, the accuracy and precision were found to be acceptable (acceptance criteria: QC medium and ULOQ ± 15% and ≤15%; QC LLOQ ± 20% and ≤20%).

Table 2
Assay performance data for niraparib, olaparib, rucaparib, talazoparib and veliparib in human plasma.

		Intra-assay (n = 15)		Inter-assay (n = 15)	
Analyte	Nominal concentration	Bias (%)	C.V. (%)	Bias (%)	C.V. (%)
Niraparib	30	± 4.2	≤2.2	−3.2	*
	1500	± 4.7	≤2.4	−4.4	*
	3000	± 3.9	≤2.6	−3.8	*
Olaparib	100	± 2.1	≤3.0	1.0	0.3
	5000	± 0.7	≤3.0	0.4	*
	10,000	± 3.3	≤3.9	−2.6	*
Rucaparib	50	± 2.1	≤4.9	−0.4	0.5
	2500	± 3.2	≤5.4	−2.8	*
	5000	± 3.1	≤3.7	−1.8	*
Talazoparib	0.5	± 6.5	≤6.5	−1.0	4.3
	25	± 6.8	≤3.7	−5.1	1.2
	50	± 5.3	≤2.1	−4.7	*
Veliparib	50	± 7.4	≤7.8	2.2	4.0
	2500	± 6.0	≤2.8	−4.1	1.6
	5000	± 4.0	≤3.9	−3.4	*

*Inter-run precision could not be calculated (mean square between group is less then mean square within groups).
C.V., coefficient of variation.

The analyte response of the lowest calibration standard was compared to the noise in a double blank sample in three analytical runs. The analyte response was at least 10 times the response in the double blank sample for all analytes, except for talazoparib. In case of talazoparib the lowest observed S/N ratio was 8. We accepted this S/N value, since the assay was optimized for this compound and the S/N ratio was still acceptable according to the FDA and EMA guidelines. Fig. 2 shows representative LC-MS/MS chromatograms of LLOQ and double blank samples.

Carry over was evaluated in three analytical runs by injecting two double blank samples after injection of the highest calibration standard. The peak area in the double blank sample should not exceed 20% of the peak area in lowest calibration standard and 5% of the peak area of the IS. No carry-over was observed for olaparib, rucaparib, talazoparib and veliparib. According to a previously published method for niraparib, carry over was not unexpected [27]. Although niraparib showed peaks in the double blank sample after injection of the highest calibration standard, peak areas were below 20%. Therefore carry-over was accepted and will not have an impact on the integrity of the data.

To investigate specificity and selectivity, six different batches of K_2EDTA plasma were spiked at LLOQ level. Double blank samples and spiked samples at LLOQ were processed and analyzed. The mean deviations from the nominal concentration and C.V. values were ≤20% for all analytes in all tested batches. No peaks were observed in the double blank samples and therefore it was concluded that no endogenous interferences were detected.

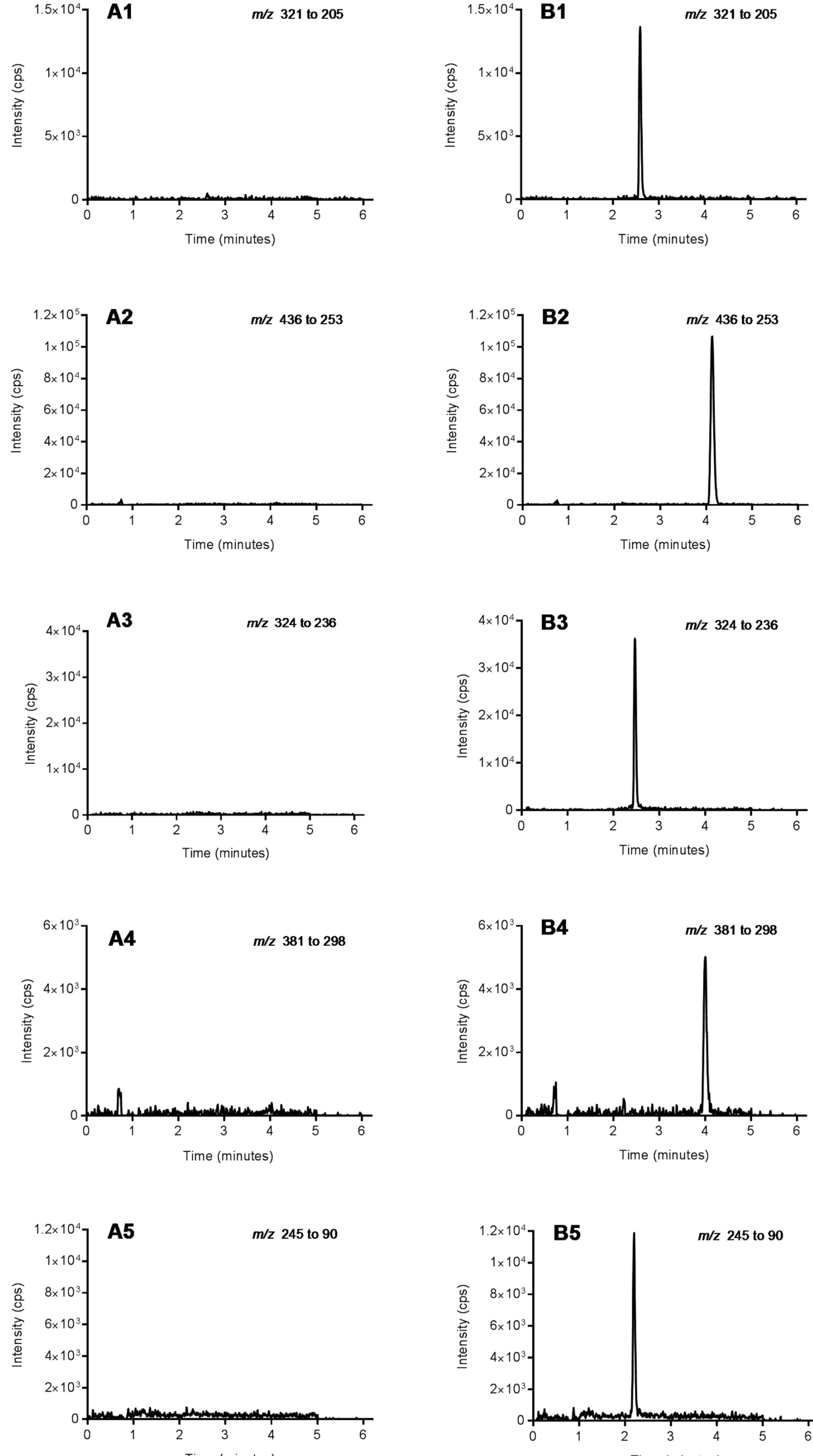


Fig. 2. Representative LC-MS/MS chromatograms of double blank- (A-series) and LLOQ (B-series) samples for niraparib (1, 30 ng/mL), olaparib (2, 100 ng/mL), rucaparib (3, 50 ng/mL), talazoparib (4, 0.5 ng/mL) and veliparib (5, 50 ng/mL).

Table 3
Stability data of niraparib, olaparib, rucaparib, talazoparib and veliparib (recovery (%) ± S.D; n = 3) in several matrices and various storing conditions (values below 80% for QC LLOQ and 85% for QC ULOQ in bold).

Matrix	Condition	Niraparib		Olaparib		Rucaparib		Talazoparib		Veliparib	
		QC LLOQ (30 ng/mL)	QC ULOQ (3000 ng/mL)	QC LLOQ (100 ng/mL)	QC ULOQ (10,000 ng/mL)	QC LLOQ (50 ng/mL)	QC ULOQ (5000 ng/mL)	QC LLOQ (0.5 ng/mL)	QC ULOQ (50 ng/mL)	QC LLOQ (50 ng/mL)	QC ULOQ (5000 ng/mL)
Human plasma	−20 °C/RT										
	3 F/T	96.6 ± 6.9	95.8 ± 3.3	101.5 ± 4.2	97.8 ± 1.9	100.0 ± 3.4	99.2 ± 1.4	93.1 ± 6.2	93.2 ± 3.7	97.8 ± 9.2	97.0 ± 1.1
	RT (light)										
	24 h	90.8 ± 3.9	91.1 ± 1.7	104.0 ± 2.5	99.4 ± 2.3	99.3 ± 1.9	99.3 ± 5.4	96.0 ± 4.3	95.5 ± 3.0	91.3 ± 3.6	92.2 ± 3.9
	48 h	85.8 ± 6.5	87.3 ± 2.1	107.0 ± 4.9	97.7 ± 3.4	102.9 ± 2.6	97.1 ± 1.4	88.7 ± 7.1	91.9 ± 2.5	**73.7 ± 2.3**	89.5 ± 2.2
	5 d	**74.7 ± 4.7**	**76.4 ± 0.7**	103.1 ± 4.0	97.4 ± 1.2	97.5 ± 8.1	97.3 ± 4.6	105.0 ± 4.0	92.2 ± 4.1	**63.5 ± 4.3**	**80.1 ± 2.5**
	RT (dark)										
	24 h	90.7 ± 3.5	90.4 ± 0.8	105.0 ± 3.3	98.8 ± 1.0	99.0 ± 3.7	100.4 ± 2.5	99.7 ± 4.0	95.9 ± 0.6	93.2 ± 2.8	91.1 ± 1.5
	48 h	83.9 ± 1.2	88.9 ± 0.6	101.6 ± 4.8	96.7 ± 3.9	102.7 ± 1.0	99.1 ± 5.8	84.1 ± 1.5	90.3 ± 3.9	80.1 ± 8.1	92.3 ± 1.8
	5 d	**77.3 ± 1.9**	**76.6 ± 2.1**	104.0 ± 3.3	97.4 ± 1.8	102.6 ± 7.4	98.3 ± 3.7	98.3 ± 6.6	93.7 ± 2.9	**65.3 ± 7.4**	**81.2 ± 2.4**
	−20 °C										
	66 d	104.8 ± 3.8	104.8 ± 1.9	112.3 ± 2.1	104.3 ± 1.1	98.9 ± 6.1	99.5 ± 0.5	113.8 ± 1.7	107.9 ± 1.1	104.1 ± 7.8	104.7 ± 1.6
	97 d	87.9 ± 5.7	104.0 ± 1.1	96.8 ± 4.0	94.5 ± 1.7	94.5 ± 4.1	96.9 ± 0.3	99.5 ± 2.6	100.4 ± 2.7	100.4 ± 14.4	103.5 ± 3.0
	152 d	89.3 ± 2.9	102.2 ± 1.4	99.3 ± 1.6	94.4 ± 2.8	97.7 ± 2.8	99.6 ± 1.9	94.7 ± 2.8	92.8 ± 1.2	103.5 ± 5.6	106.6 ± 4.7
Final extract	2–8 °C										
	5 d	98.2 ± 1.3	97.0 ± 1.7	100.7 ± 4.1	96.8 ± 1.3	101.9 ± 4.5	94.6 ± 2.0	108.1 ± 5.1	94.6 ± 1.3	103.5 ± 2.7	98.4 ± 2.2
	66 d	96.0 ± 3.5	101.9 ± 1.8	97.7 ± 6.6	101.7 ± 2.9	92.6 ± 4.9	98.1 ± 1.4	100.6 ± 3.1	104.6 ± 1.8	102.7 ± 4.3	96.1 ± 1.2
	97 d	95.3 ± 6.2	101.8 ± 3.4	95.3 ± 3.0	87.0 ± 2.1	97.0 ± 1.4	96.9 ± 1.0	97.8 ± 3.6	96.7 ± 3.9	107.3 ± 4.7	104.2 ± 1.1

RT, room temperature; F/T, freeze-thaw cycles; h, hours; d, days

Various stability conditions were tested in triplicate at QC LLOQ and QC ULOQ level. Analytes were considered stable in human plasma or final extract if 85–115% of the initial concentration of QC ULOQ level and 80–120% of the initial concentration of QC LLOQ level was recovered. QC samples kept under various stability conditions were quantified on freshly prepared calibration standards. Data on stability in human plasma and final extract are shown in Table 3. Analytes were not sensitive to light exposure, since no differences were observed in recovery between samples kept in the dark and light. Olaparib, rucaparib and talazoparib were stable at room temperature for at least 5 days and niraparib was stable for 48 h at room temperature. Veliparib was only stable for 24 h which means patient samples should be handled quickly and shipped on ice if transport takes longer than 24 h. Previous developed methods for niraparib or veliparib tested stability only up to 4–6 h [20,21,26,27], which was not informative enough, since shipping of samples can take longer.

Stock solutions were considered stable when 95–105% of the concentration was recovered. Stock solutions of niraparib, talazoparib and veliparib in DMSO were stable for at least 418 days at −70 °C. The stock solution of rucaparib in DMSO was stable for at least 413 days at −70 °C and the stock solution of olaparib in DMSO-methanol (20:80, v/v) was stable for at least 2213 days at −20 °C. Reinjection reproducibility was tested and showed the entire analytical run can be reanalyzed after 8 days when kept at 2–8 °C.

3.3. *Clinical application*

The assay was used to determine plasma concentrations of patients treated with olaparib and niraparib to show the applicability of the assay. Ten patients were included for each drug and the mean measured plasma concentration in these ten patients was 2691 ng/mL for olaparib and 507 ng/mL for niraparib (Table 4). Representative LC-MS/MS chromatograms of plasma from a patient treated with niraparib and a patient treated with olaparib are depicted in Fig. 3. All measured olaparib and niraparib concentrations were within the validated range. Previous published methods on the quantification of olaparib and niraparib would not have covered high concentrations in patient samples, without a dilution step [19,22,27]. Since our assay will be used for routine measurement and fast results are essential, the advantage of our developed assay is the ability to measure concentrations of olaparib and niraparib over the entire range of C_{min} to C_{max} without any need for dilution.

4. Conclusion

We developed an new LC-MS/MS assay for the simultaneously quantification of the PARP-inhibitors niraparib, olaparib, rucaparib, talazoparib and veliparib for TDM purposes. To our knowledge, this is the first assay for the combined analysis of the five PARP inhibitors and the first assay for quantification of talazoparib in human plasma, The assay was successfully validated using a TDM validation approach. The validated range was 30–3000 ng/mL for niraparib, 100–10,000 ng/mL for olaparib, 50–5000 ng/mL for rucaparib, 0.5–50 ng/mL for talazoparib and 50–5000 for veliparib. General mass spectrometric parameters were optimized for talazoparib to obtain sufficient sensitivity at LLOQ level for this drug and analytes specific parameters were de-optimized for niraparib, rucaparib, olaparib and veliparib to prevent detector saturation. Chromatographic separation of olaparib and talazoparib was necessary to obtain un-biased concentration determinations for talazoparib. In conclusion, the first combined assay for PARP-inhibitors was developed and successfully validated. The assay has been implemented to measure plasma concentrations of patients using niraparib and olaparib for TDM and exposure-response studies. Additionally, the assay will be used in the near future to facilitate TDM and exposure-response studies for the other analytes.

Table 4
Recommended dose by the EMA [34,35], validated concentration range and measured plasma concentrations of patients using niraparib or olaparib (n = 10).

Analyte	Recommended dose (mg)	Validated concentration range (ng/mL)	Mean measured plasma concentration (ng/mL)	Measured plasma concentration range (ng/mL)
Niraparib	300 mg OD*	30–3000	507	182–1200
Olaparib	300 mg BID** (tablets) 400 mg BID (capsules)	100–10,000	2691	854–5510

*OD: once-daily
**BID: twice-daily.

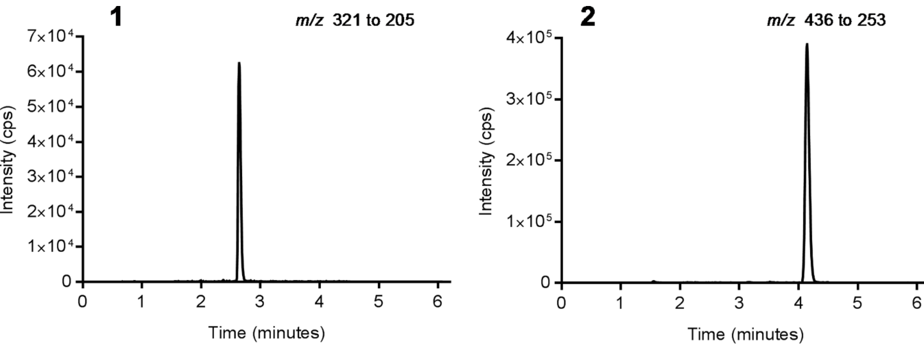


Fig. 3. Representative LC-MS/MS chromatograms of plasma from (1) a patient using niraparib (431 ng/mL) and (2) a patient using olaparib (1660 ng/mL).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

M.A.C. Bruin: Methodology, Investigation, Validation, Formal analysis, Writing - original draft. **N. de Vries:** Project administration, Writing - review & editing. **L. Lucas:** Project administration, Writing - review & editing. **H. Rosing:** Conceptualization, Supervision, Writing - review & editing. **A.D.R. Huitema:** Supervision, Writing - review & editing. **J.H. Beijnen:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2019.121925.

References

[1] I. Faraoni, G. Graziani, Role of BRCA mutations in cancer treatment with poly(ADP-ribose) polymerase (PARP) inhibitors, Cancers (Basel) 10 (2018) 487, https://doi.org/10.3390/cancers10120487.
[2] Y. Chen, H. Du, The promising PARP inhibitors in ovarian cancer therapy: from Olaparib to others, 2018. doi: 10.1016/j.biopha.2018.01.094.
[3] S. Tangutoori, P. Baldwin, S. Sridhar, PARP inhibitors: a new era of targeted therapy, Maturitas 81 (2015) 5–9, https://doi.org/10.1016/j.maturitas.2015.01.015.
[4] T.K. Ricks, H.-J. Chiu, G. Ison, G. Kim, A.E. McKee, P. Kluetz, R. Pazdur, Successes and challenges of PARP inhibitors in cancer therapy, Front. Oncol. 5 (2015) 1–5, https://doi.org/10.3389/fonc.2015.00222.
[5] K.E. McCann, S.A. Hurvitz, Advances in the use of PARP inhibitor therapy for breast cancer, Drugs Context. 7 (2018) 1–30, https://doi.org/10.7573/dic.212540.
[6] N. Widmer, C. Bardin, E. Chatelut, A. Paci, J. Beijnen, D. Levêque, G. Veal, A. Astier, C. Bardin, D. Levêque, N. Widmer, J. Beijnen, A. Astier, E. Chatelut, Review of therapeutic drug monitoring of anticancer drugs part two – targeted therapies, Eur. J. Cancer 50 (2014) 2020–2036, https://doi.org/10.1016/j.ejca.2014.04.015.
[7] J.J.J. Geenen, S.C. Linn, J.H. Beijnen, J.H.M. Schellens, PARP inhibitors in the treatment of triple-negative breast cancer, Clin. Pharmacokinet. 57 (2018) 427–437, https://doi.org/10.1007/s40262-017-0587-4.
[8] US Food and Drug Administration (FDA), TALZENNA (talazoparib): US prescribing information, 2018, pp. 1–17. < https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/211651s000lbl.pdf > (accessed June 21, 2019).
[9] Committee for Medicinal Products for Human Use (CHMP) European Medicines Agency (EMA), ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS - Olaparib, 2019, pp. 1–87. < https://www.ema.europa.eu/en/documents/product-information/lynparza-epar-product-information_en.pdf > (accessed July 22, 2019).
[10] Committee for Medicinal Products for Human Use (CHMP) European Medicines Agency (EMA), ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS - Niraparib, 2017, pp. 1–32. < https://www.ema.europa.eu/en/documents/product-information/zejula-epar-product-information_en.pdf > (accessed June 21, 2019).
[11] Committee for Medicinal Products for Human Use (CHMP) European Medicines Agency (EMA), ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS - Rucaparib, 2019, pp. 1–79. < https://www.ema.europa.eu/en/documents/product-information/rubraca-epar-product-information_en.pdf > (accessed June 21, 2019).
[12] X. Li, J. Delzer, R. Voorman, S.M. De Morais, Y. Lao, Disposition and drug-drug interaction potential of veliparib (ABT-888), a novel and potent inhibitor of poly (ADP-ribose) polymerase, Drug Metab. Dispos. 39 (2011) 1161–1169, https://doi.org/10.1124/dmd.110.037820.
[13] C. Pressiat, H.-H. Huynh, A. Plé, H. Sauvageon, I. Madelaine, C. Chougnet, C. Le Maignan, S. Mourah, L. Goldwirt, Development and validation of a simultaneous quantification method of ruxolitinib, vismodegib, olaparib, and pazopanib in human plasma using liquid chromatography coupled with tandem mass spectrometry, Ther. Drug Monit. 40 (2018) 337–343, https://doi.org/10.1097/FTD.0000000000000497.
[14] R.B. Verheijen, H. Yu, J.H.M. Schellens, J.H. Beijnen, N. Steeghs, A.D.R. Huitema, Practical recommendations for therapeutic drug monitoring of kinase inhibitors in oncology, Clin. Pharmacol. Ther. 102 (2017), https://doi.org/10.1002/cpt.787.
[15] US Food and Drug Administration (FDA), CENTER FOR DRUG EVALUATION AND RESEARCH-MULTI-DISCIPLINE REVIEW, Olaparib, 2016, pp. 1–233. < https://www.fda.gov/Drugs/ > (accessed June 21, 2019).
[16] US Food and Drug Administration (FDA), Center for drug evaluation-multi-discipline review, Niraparib, 2016.
[17] US Food and Drug Administration (FDA), Center for drug evaluation and research-multi-discipline review, Rucaparib (2016) 1–232. < https://www.fda.gov/Drugs/ > (accessed June 21, 2019).
[18] US Food and Drug Administration (FDA), Center for drug evaluation and research-multi-discipline review, Talazoparib, (2018) 1–199. < https://www.fda.gov/Drugs/ > (accessed June 21, 2019).
[19] C.M. Nijenhuis, L. Lucas, H. Rosing, J.H.M. Schellens, J.H. Beijnen, Development and validation of a high-performance liquid chromatography–tandem mass spectrometry assay quantifying olaparib in human plasma, J. Chromatogr. B. 940 (2013) 121–125, https://doi.org/10.1016/j.jchromb.2013.09.020.
[20] S. Reinhardt, M. Zhao, A. Mnatsakanyan, L. Xu, R.M. Ricklis, A. Chen, J.E. Karp, M.A. Rudek, A rapid and sensitive method for determination of veliparib (ABT-888), in human plasma, bone marrow cells and supernatant by using LC/MS/MS, J. Pharm. Biomed. Anal. 52 (2010) 122–128, https://doi.org/10.1016/j.jpba.2009.12.015.
[21] R.A. Parise, M. Shawaqfeh, M.J. Egorin, J.H. Beumer, Liquid chromatography–mass

spectrometric assay for the quantitation in human plasma of ABT-888, an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase, J. Chromatogr. B. 872 (2008) 141–147, https://doi.org/10.1016/j.jchromb.2008.07.032.

[22] J. Roth, C. Peer, B. Mannargudi, H. Swaisland, J.-M. Lee, E. Kohn, W. Figg, A sensitive and robust ultra HPLC assay with tandem mass spectrometric detection for the quantitation of the PARP inhibitor olaparib (AZD2281) in human plasma for pharmacokinetic application, Chromatography 1 (2014) 82–95, https://doi.org/10.3390/chromatography1020082.

[23] R.W. Sparidans, S. Durmus, A.H. Schinkel, J.H.M. Schellens, J.H. Beijnen, Liquid chromatography-tandem mass spectrometric assay for the PARP inhibitor rucaparib in plasma, J. Pharm. Biomed. Anal. (2014), https://doi.org/10.1016/j.jpba.2013.10.016.

[24] L. Ye, J. Chen, S. Li, Y. Zhu, S. Xie, X. Du, UPLC-MS/MS method for the determination of talazoparib in rat plasma and its pharmacokinetic study, J. Pharm. Biomed. Anal. 177 (2020) 112850, , https://doi.org/10.1016/j.jpba.2019.112850.

[25] V. Gorijavolu, A sensitive bio analytical method development and validation of rucaparib in human plasma by LC-ESI-MS/MS, Int. J. Adv. Res. 6 (2018) 836–843, https://doi.org/10.21474/IJAR01/6290.

[26] R. Wiegand, J. Wu, X. Sha, P. LoRusso, J. Li, Simultaneous determination of ABT-888, a poly (ADP-ribose) polymerase inhibitor, and its metabolite in human plasma by liquid chromatography/tandem mass spectrometry, J. Chromatogr. B 878 (2010) 333–339, https://doi.org/10.1016/j.jchromb.2009.11.037.

[27] L. Van Andel, Z. Zhang, S. Lu, V. Kansra, S. Agarwal, L. Hughes, M.M. Tibben, A. Gebretensae, H. Rosing, J.H.M.M. Schellens, J.H. Beijnen, Liquid chromatography-tandem mass spectrometry assay for the quantification of niraparib and its metabolite M1 in human plasma and urine, J. Chromatogr. B 1040 (2017) 14–21, https://doi.org/10.1016/j.jchromb.2016.11.020.

[28] R.W. Sparidans, I. Martens, L.B.J. Valkenburg-van Iersel, J. den Hartigh, J.H.M. Schellens, J.H. Beijnen, Liquid chromatography–tandem mass spectrometric assay for the PARP-1 inhibitor olaparib in combination with the nitrogen mustard melphalan in human plasma, J. Chromatogr. B. 879 (2011) 1851–1856, https://doi.org/10.1016/j.jchromb.2011.05.003.

[29] J. Jolibois, A. Schmitt, B. Royer, A simple and fast LC-MS/MS method for the routine measurement of cabozantinib, olaparib, palbociclib, pazopanib, sorafenib, sunitinib and its main active metabolite in human plasma, J. Chromatogr. B Anal. Technol. Biomed Life Sci. 1132 (2019) 121844, https://doi.org/10.1016/j.jchromb.2019.121844.

[30] M. van Nuland, H. Rosing, J.H.M. Schellens, J.H. Beijnen, Bioanalytical LC-MS/MS validation of therapeutic drug monitoring assays in oncology, Biomed. Chromatogr. e4623 (2019), https://doi.org/10.1002/bmc.4623.

[31] M. van Nuland, N. Venekamp, N. de Vries, K.A.M. de Jong, H. Rosing, J.H. Beijnen, Development and validation of an UPLC-MS/MS method for the therapeutic drug monitoring of oral anti-hormonal drugs in oncology, J. Chromatogr. B Anal. Technol. Biomed Life Sci. 1106–1107 (2019) 26–34, https://doi.org/10.1016/j.jchromb.2019.01.001.

[32] US Food and Drug Administration (FDA), Guidance for industry: bioanalytical method validation guidance for industry bioanalytical method validation (2018) 1–44. < http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm > (accessed April 4, 2019).

[33] European Medicines Agency (EMA), Guideline on bioanalytical method validation (2011) 1–23. < https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf > (accessed April 24, 2019).

[34] European Medicines Agency (EMA), CHMP assessment report on extension of marketing authorisation grouped with a variation, Lynparza (2018) 1–163. < https://www.ema.europa.eu/en/documents/variation-report/lynparza-h-c-3726-x-0016-g-epar-assessment-report-extension_en.pdf > (accessed June 21, 2019).

[35] European Medicines Agency (EMA), Zejula Public Assessment Report (2017) 1–122. < https://www.ema.europa.eu/en/documents/assessment-report/zejula-epar-public-assessment-report_en.pdf > (accessed July 18, 2019).

[36] European Medicines Agency (EMA), Rubraca Public Assessment Report (2018) 1–167. < https://www.ema.europa.eu/en/documents/assessment-report/rubraca-epar-public-assessment-report_en.pdf > (accessed July 18, 2019).

[37] European Medicines Agency (EMA), Talzenna Public Assessment report (2019) 1–140. < https://www.ema.europa.eu/en/documents/assessment-report/talzenna-epar-public-assessment-report_en.pdf > (accessed July 18, 2019).

[38] J. Mateo, V. Moreno, A. Gupta, S.B. Kaye, E. Dean, M.R. Middleton, M. Friedlander, C. Gourley, R. Plummer, G. Rustin, C. Sessa, K. Leunen, J. Ledermann, H. Swaisland, A. Fielding, W. Bannister, S. Nicum, L.R. Molife, An adaptive study to determine the optimal dose of the tablet formulation of the PARP inhibitor olaparib, Target. Oncol. 11 (2016) 401–415, https://doi.org/10.1007/s11523-016-0435-8.

[39] G.I. Shapiro, R.S. Kristeleit, H.A. Burris, P. Lorusso, M.R. Patel, Y. Drew, H. Giordano, L. Maloney, S. Watkins, S. Goble, S. Jaw-Tsai, J.J. Xiao, Pharmacokinetic study of rucaparib in patients with advanced solid tumors, Clin. Pharmacol. Drug Dev. n.d (2019), https://doi.org/10.1002/cpdd.575.

[40] J. de Bono, R.K. Ramanathan, L. Mina, R. Chugh, J. Glaspy, S. Rafii, S. Kaye, J. Sachdev, J. Heymach, D.C. Smith, J.W. Henshaw, A. Herriott, M. Patterson, N.J. Curtin, L.A. Byers, Z.A. Wainberg, Phase I, dose-escalation, two-part trial of the PARP inhibitor talazoparib in patients with advanced germline BRCA1/2 mutations and selected sporadic cancers, Cancer Discov. 7 (2017) 620–629, https://doi.org/10.1158/2159-8290.CD-16-1250.

[41] T. Nishikawa, K. Matsumoto, K. Tamura, H. Yoshida, Y. Imai, A. Miyasaka, T. Onoe, S. Yamaguchi, C. Shimizu, K. Yonemori, T. Shimoi, M. Yunokawa, H. Xiong, S. Nuthalapati, H. Hashiba, T. Kiriyama, T. Leahy, P. Komarnitsky, K. Fujiwara, Phase 1 dose-escalation study of single-agent veliparib in Japanese patients with advanced solid tumors, Cancer Sci. 108 (2017) 1834–1842, https://doi.org/10.1111/cas.13307.

[42] T.L. Werner, J. Sachdev, E.M. Swisher, M. Gutierrez, M. Kittaneh, M.N. Stein, H. Xiong, M. Dunbar, D. Sullivan, P. Komarnitsky, M. McKee, A.R. Tan, Safety and pharmacokinetics of veliparib extended-release in patients with advanced solid tumors: a phase I study, Cancer Med. 7 (2018) 2360–2369, https://doi.org/10.1002/cam4.1488.

[43] M.K. Hidau, S. Kolluru, S. Palakurthi, Development and validation of a high performance liquid chromatography method for the quantification of talazoparib in rat plasma: application to plasma protein binding studies, Biomed. Chromatogr. 32 (2018) e4046, , https://doi.org/10.1002/bmc.4046.

[44] C. Polson, P. Sarkar, B. Incledon, V. Raguvaran, R. Grant, Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 785 (2003) 263–275, https://doi.org/10.1016/S1570-0232(02)00914-5.

[45] 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. 32 (2018) e4147, , https://doi.org/10.1002/bmc.4147.