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Short communication

Liquid chromatography–tandem mass spectrometric assay for the PARP inhibitor rucaparib in plasma

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ABSTRACT

A quantitative bioanalytical liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay for the poly(ADP-ribose) polymerase-1 inhibitor rucaparib was developed and validated. Plasma samples were pre-treated using protein precipitation with acetonitrile containing gefitinib as internal standard. Diluted extract was directly injected into the reversed-phase chromatographic system. The eluate was transferred into the electrospray interface with positive ionization and the analyte was detected in the selected reaction monitoring mode of a triple quadrupole mass spectrometer.

The assay was validated in a 1.25–2000 ng/ml calibration range with $r^2 = 0.9958 \pm 0.0012$ for linear regression with quadratic weighting ($n = 6$). Within day precisions ($n = 18$) were 2.0–5.4%, between day (3 days; $n = 18$) precisions 3.2–8.0% and accuracies ($n = 18$) were 89.7–93.2%. At the lower limit of quantification (1.25 ng/ml) these parameters were 9.6%, 13.7% and 85.3%, respectively. The drug was sufficiently stable under all relevant analytical conditions. Finally, the assay was successfully used to determine drug pharmacokinetics in female FVB wild type mice.

1. Introduction

Rucaparib (AG014699; Fig. 1) is an inhibitor of poly (ADP-ribose) polymerase-1 (PARP-1). This polymerase plays a key role in DNA repair, especially base excision repair (BER), and is demonstrated to show enhanced expression in different cancer cell lines [1]. PARP-1 knockout mouse models showed only *ca.* 10% remaining PARP activity, due to PARP-2. PARP inhibitors can enhance the efficacy of DNA-double strand breaks inducing cytotoxic and radiation therapies based on DNA damage in several tumor types and are particularly cytotoxic against tumors in BRCA1 or BRCA2 mutation carriers. Therefore, most clinical studies on PARP inhibitors are based on combination therapies. Very recently, the first Phase II study with intravenous rucaparib, in combination with oral temozolomide, showed an increase in progression-free survival for patients with metastatic melanoma [2]. Currently, studies use most often oral rucaparib monotherapy or combinations with intravenous cisplatin or carboplatin on BRCA mutated breast and ovarian cancer and other solid tumors [3]. Despite the number of studies being performed on patients, biotransformation routes and validated bioanalytical assays have not been reported for rucaparib, hitherto. Therefore, we developed and validated a bioanalytical LC–MS/MS assay for this drug. The applicability of the assay was demonstrated in a pharmacokinetic mouse study.

2. Experimental

2.1. Chemicals

Rucaparib (phosphate salt; >98.5%) and gefitinib were purchased from Sequoia Research Products (Pangbourne, UK). Water (LC–MS grade), methanol (HPLC grade) and acetonitrile (HPLC-S grade) were obtained from Biosolve (Valkenswaard, The Netherlands). Water, not used as eluent, was home purified by reversed osmosis on a multi-laboratory scale. Formic acid was of analytical grade originating from Merck (Darmstadt, Germany) and analytical grade dimethyl sulfoxide (DMSO) was supplied by Acros Organics (Geel, Belgium). Human EDTA disodium plasma (pooled and from individual donors) and pooled female lithium

Abbreviations: AUC, area under the plasma concentration-time curve; LLOQ, lower limit of quantification; PARP, poly(ADP-ribose) polymerase; SRM, selected reaction monitoring; $T_{1/2}$, elimination half-life; QC, quality control.

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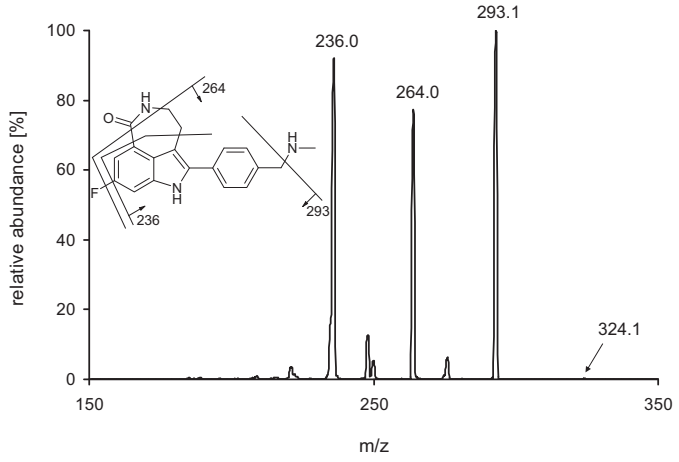

Fig. 1. Chemical structure and product spectrum, formed by collision induced dissociation of the protonated molecules of rucaparib, *m*/*z* 324.15@-28 V.

heparin mouse plasma originated from Sera Laboratories International (Haywards Heath, UK).

Stock solutions of rucaparib (phosphate salt) at 0.25 mg/ml were prepared in methanol. Gefitinib was prepared at 5 mg/ml in DMSO.

2.2. Equipment

The LC–MS/MS equipment consisted of a DGU-14A degasser, a CTO-10Avp column oven, a Sil-HTc autosampler and two LC10-ADvp-μ pumps (all from Shimadzu, Kyoto, Japan) and a Finnigan TSQ Quantum Discovery Max triple quadrupole mass spectrometer with electrospray ionization (Thermo Electron, Waltham, MA, USA). Data were recorded on and the system was controlled using the Finnigan Xcalibur software (version 1.4, Thermo Electron).

2.3. LC–MS/MS conditions

Partial-loop injections (2 μl) were made on a Polaris 3 C18-A column (50 × 2 mm, d_p = 3 μm, average pore diameter = 10 nm, Varian, Middelburg, The Netherlands) with a corresponding pre-column (10 × 2 mm). The column temperature was maintained at 40 °C and the sample rack compartment at 4 °C. A gradient (0.5 ml/min) using 0.02% (v/v) formic acid (A) and methanol (B) was used. After injection, the percentage of methanol was increased linearly from 25 to 40% (v/v) during 1.33 min. Next, the column was flushed with 100% (v/v) methanol for 0.67 min and finally, the column was reconditioned at the starting conditions (25% (v/v) B) for 1 min, resulting in a total run time of 3 min. The whole eluate was transferred into the electrospray probe, starting at 0.8 min after injection by switching the MS divert valve until 2.2 min after injection. The electrospray was tuned in the positive ionization mode by introducing 0.5 ml/min of a solvent mixture (methanol and 0.1% formic acid; 1:1 (v/v)) and 5 μl/min of 10 μg/ml of rucaparib. Electrospray settings of the assay were a 4400 V spray voltage, a 380 °C capillary temperature and the nitrogen sheath, ion sweep and auxiliary gasses were set at 41, 10 and 21 arbitrary units, respectively; the skimmer voltage was set at −8 V. The SRM mode was used with argon as the collision gas at 1.3 mTorr. The tube lens off set was 92 V for rucaparib and 119 V for gefitinib. Rucaparib was monitored at *m*/*z* 324.1 → 293; 236 at −17 and −36 V collision energies, respectively, with 0.2 s dwell times and gefitinib at *m*/*z* 447.1 → 128 at −22 V with a 0.1 s dwell time. Mass resolutions were set at 0.7 full width at half height (unit resolution) for both separating quadrupoles.

2.4. Sample pre-treatment

To a 50 μl plasma sample, pipetted into a polypropylene reaction tube, 75 μl of 89 ng/ml gefitinib in acetonitrile was added. The tube was closed and shaken by vortex mixing for 5–10 s. After centrifugation of the sample at 10,000 × *g* at 20 °C for 1 min, 100 μl of the supernatant was transferred to a 250 μl glass insert placed in an auto-injector vial. Before closing the vial, 100 μl of water was added and finally, 2 μl of the mixture was injected onto the column.

2.5. Validation

A laboratory scheme based on international guidelines was used for the validation procedures [4].

2.5.1. Calibration

One rucaparib stock solution (0.25 mg/ml of phosphate salt) was diluted to a 2000 ng/ml calibration solution in human plasma. All solutions were stored in a 1.5-ml polypropylene tube at −30 °C. Additional calibration samples were prepared daily at 500, 100, 25, 5 and 1.25 ng/ml by dilution with blank plasma. The calibration samples were processed in duplicate for each daily calibration. Weighted linear least-squares regression with $1/X^2$ (reversed squared concentration) as the weighting factor was employed to define the calibration curves using the ratios of the peak area of the analyte and the IS.

2.5.2. Precision and accuracy

A second stock solution of rucaparib phosphate was used to obtain validation (quality control; QC) samples in pooled human EDTA disodium plasma at 1500 (QC-high), 75 (QC-med), 3.75 (QC-low) and 1.25 ng/ml (QC-LLOQ). The samples were stored in polypropylene tubes at −30 °C. Precisions and accuracies were determined by sextuple analysis of each QC in three analytical runs on three separate days for all QCs (total: n = 18 per QC). Relative standard deviations were calculated for both, the within and between day precisions.

2.5.3. Selectivity

Six individual human plasma samples were processed to test the selectivity of the assay. The samples were processed without rucaparib and IS and with rucaparib at the LLOQ level (1.25 ng/ml), supplemented with the IS.

2.5.4. Recovery and matrix effect

The recovery was determined (n = 4) by comparing processed samples (QC-high, -med, -low) with reference rucaparib solutions in blank plasma extract at the same levels. The matrix effect was assessed by comparing the reference solutions in blank human plasma extracts with corresponding matrix free solutions at the three validation levels. An analogous procedure was used for the internal standard.

2.5.5. Stability

The stability of rucaparib was investigated in QC-high and -low plasma samples stored in polypropylene tubes. Quadruplicate analysis of these samples from separate tubes was performed after storage at 20 °C (ambient temperature) for 24 h, three additional freeze–thaw cycles (thawing at 20 °C during *ca.* 2 h and freezing again at −30 °C for at least one day), and storage at −30 °C for 3 months, respectively. Furthermore, QC samples were re-injected together with fresh calibration samples after additional storage of the extracts at 4 °C for three nights to test the stability at the conditions in the auto-injector.

Finally, the responses of rucaparib from the stock solutions in methanol after 6 h at 20 °C (n = 2) and after 2 months at −30 °C