Journal of Pharmaceutical and Biomedical Analysis 88 (2014) 216–220

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Liquid chromatography–tandem mass spectrometric assay for the cyclin-dependent kinase inhibitor AT7519 in mouse plasma

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ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form 30 August 2013
Accepted 31 August 2013
Available online 12 September 2013

Keywords:
AT7519
Cyclin-dependent kinase inhibitor
LC–MS/MS
Mouse plasma

ABSTRACT

A quantitative bioanalytical liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay for the cyclin-dependent kinase inhibitor AT7519 in mouse plasma was developed and validated. Plasma samples were pre-treated using protein precipitation with acetonitrile containing rucaparib as internal standard. After dilution with water, the extract was directly injected into the reversed-phase LC 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 5–10,000 ng/ml calibration range using double logarithmic calibration, 5 ng/ml was the lower limit of quantification. Within day precisions ($n = 6$) were 2.9–5.6%, between day (3 days; $n = 18$) precisions 3.2–7.2%. Accuracies were between 95.9 and 99.0% for the whole calibration range. The drug was stable under all relevant analytical conditions. Finally, the assay was successfully used to determine plasma pharmacokinetics after intraperitoneal administration of AT7519 in mice with neuroblastoma xenografts.

1. Introduction

Neuroblastoma is the most commonly diagnosed type of extracranial cancer in young children and the survival rate for patients with high stage neuroblastoma is only 30–40% [1]. AT7519 (Fig. 1) is a recently developed CDK2 (cyclin-dependent kinase 2) inhibitor and is a promising drug candidate for the treatment of high risk neuroblastoma patients with MYCN (V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)) amplification. MYCN amplification occurs in 20–30% of all high risk neuroblastoma patients [2] and MYCN-overexpressing neuroblastoma cells have shown to be sensitive to CDK2 inactivation [3].

For the clinical implementation of AT7519, it is important to perform preclinical animal studies to evaluate if plasma AT7519 levels correlate with efficacy and toxicity and therefore can be used to tailor drug doses to individual patients. Thus, the availability of a sensitive method for the analysis of AT7519 plasma levels is necessary. Currently, two studies addressing AT7519 pharmacokinetics and -dynamics reported the analysis of AT7519 levels in, respectively, mouse [4] and human [5] plasma by LC–MS/MS. Unfortunately, information about the analytical conditions is very limited for the human plasma assay using liquid-liquid extraction [5] and almost nil for the mouse plasma assay [4].

Therefore, we now report the development and validation of a novel bioanalytical assay for determination of AT7519 levels in mouse plasma, using LC–MS/MS and protein precipitation as a simple pre-treatment procedure. This assay is a valuable tool to support preclinical studies with AT7519.

2. Experimental

2.1. Chemicals

AT7519 (>98%) was kindly supplied by Astex (Cambridge, UK) and rucaparib (phosphate salt; >98.5%; internal standard (IS)) was purchased from Sequoia Research Products (Pangbourne, UK).

Abbreviations: $AUC_{0-\infty}$, area under the plasma concentration–time curve; CDK, cyclin-dependent kinase; C_{max}, maximum plasma concentration; EDTA, ethylenediaminetetraacetic acid; i.p., intraperitoneal; IS, internal standard; MYCN, V-myc myelocytomatosis viral related oncogene neuroblastoma derived (avian); $T_{1/2}$, elimination half-life; T_{max}, time to C_{max}.

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http://dx.doi.org/10.1016/j.jpba.2013.08.051

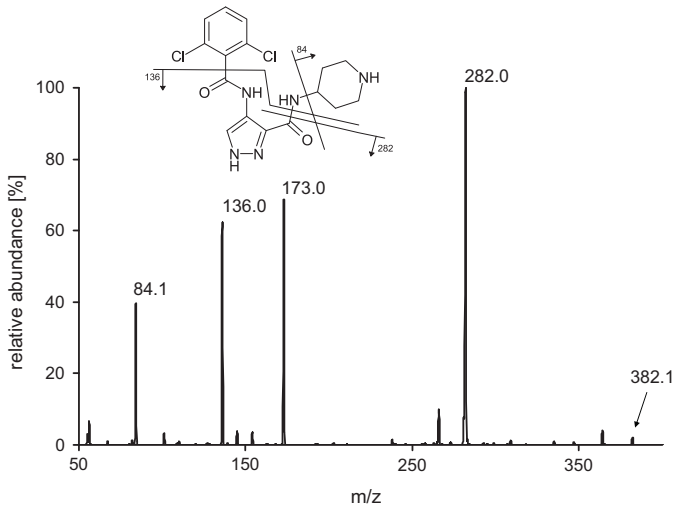

Fig. 1. Chemical structure and product spectrum, formed by collision induced dissociation of the protonated molecule of AT7519, *m/z* 382.1@-30 V.

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). Mouse potassium ethylenediaminetetraacetic acid (EDTA) plasma was supplied by Seralab Laboratories (Haywards Heath, UK).

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 (5 μl) were made on a Polaris 3 C18-A column (50 mm × 2 mm, d_p = 3 μm, average pore diameter = 10 nm, Varian, Middelburg, The Netherlands) with a corresponding pre-column (10 mm × 2 mm). The column temperature was maintained at 50 °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 20 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 (20% (v/v) B) for 1 min resulting in a total run time of 3 min. The eluate was only introduced into the MS from 0.8 min to 2.2 min by using a divert valve. The electrospray was tuned in the positive ionization mode by introducing 0.5 ml/min of a solvent mixture of 50% (v/v) methanol and 50% (v/v) of 0.1% (v/v) formic acid in water, mixed with 5 μl/min of 10 μg/ml AT7519. Electrospray settings of the assay were a 4500 V spray voltage, a 391 °C capillary temperature and the nitrogen sheath, ion sweep and auxiliary gasses were set at 45, 0 and 8 arbitrary units, respectively; the skimmer voltage was set at −5 V. The SRM mode was used with argon as the collision gas at 1.5 mTorr. The tube lens off set was 130 V for AT7519 and 92 V for rucaparib. AT7519 was monitored at *m/z* 382.1 → 135.9; 281.9 at −40 and −25 V collision energies, respectively, with 0.2 s dwell times and the IS rucaparib at *m/z* 324.1 → 236.0; 293.0 at −36 and −17 V with 0.05 s dwell times. 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 volume of 20 μl of mouse plasma, pipetted into a polypropylene reaction tube, 30 μl of 50 ng/ml rucaparib in acetonitrile were added. The tubes were closed and shaken by vortex mixing for *ca.* 5 s. After centrifugation of the sample at 10,000 × *g* at 20 °C for 1 min, 40 μl of the supernatant was transferred to a 250 μl glass insert placed in an autoinjector vial. Before closing the vial, 100 μl of water was added and finally, 5 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 [6].

2.5.1. Calibration

Stock solutions of AT7519 at 1 and 0.5 mg/ml were prepared in methanol. Rucaparib was prepared at 0.25 mg/ml in methanol. The 1 mg/ml AT7519 stock solution was diluted to a 10,000 ng/ml calibration solution in potassium EDTA mouse plasma. All solutions were stored in a 1.5-ml polypropylene tube at −30 °C. Additional calibration samples were prepared daily at 5000, 1000, 500, 100, 50, 10 and 5 ng/ml by dilution with blank mouse plasma. The highest and two lowest calibration samples were processed in duplicate for each daily calibration, whereas the levels in between were processed only once. Least-squares double logarithmic linear regression 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

The 0.5 mg/ml stock solution of AT7519 was used to obtain validation (quality control; QC) samples in pooled mouse potassium EDTA plasma at 7500 (QC-high), 300 (QC-med), 15 (QC-low) and 5 ng/ml (QC-LLOQ). 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 mouse plasma samples were processed to test the selectivity of the assay. Samples were processed without AT7519 and IS and with AT7519 at the LLOQ level (5 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 AT7519 solutions in blank plasma extract at the same levels. The matrix effect was assessed by comparing the reference solutions in blank plasma extracts with the same matrix free solutions at the three validation levels. An analogous procedure was used for the IS rucaparib.

2.5.5. Stability

The stability of AT7519 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