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Quantification and clinical validation of the selective MET kinase inhibitor DO-2 and its metabolites DO-5 and M3 in human plasma

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ABSTRACT

DO-2 is a highly selective MNNG HOS transforming (MET) inhibitor. This deuterated drug is thought to diminish the formation of the Aldehyde Oxidase 1 inactive metabolite M3. For various reasons, quantification of DO-2 and its metabolites M3 and DO-5 is highly relevant. In this study, we present an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method to quantify DO-2, M3 and DO-5. Rolipram served as the internal standard. Aliquots of 25 μL were mixed with 100 μL internal standard consisting of 10 ng/mL rolipram in acetonitrile. Separation of the analytes was achieved on an Acquity UPLC ® HSS T3 column, utilizing gradient elution with water/formic acid and acetonitrile/formic acid at a flow-rate of 0.400 mL/min. Calibration curves were linear in the range of 1.00 – 1000 ng/mL for DO-2 and DO-5, and 2.00 – 2000 ng/mL for M3 in human plasma. The within-run and between-run precisions of DO-2, DO-5 and M3, also at the level of the LLQ, were within 12.1%, while the accuracy ranged from 89.5 to 108.7%. All values for accuracy, within-run and between-run precisions met the criteria set by the Food and Drug Administration. The method was effectively employed in the analysis of samples obtained from a clinical trial.

1. Introduction

The MNNG HOS transforming (MET) proto-oncogene encodes for the receptor tyrosine kinase MET. Activating mutations in the MET kinase domain have been identified in certain cancer types, particularly in hereditary and sporadic papillary kidney cancer [1–4]. These findings substantiate the role of dysfunction of the MET signaling pathway in tumorigenesis. Moreover, MET exon 14 skipping mutations in non-small lung cancer (NSCLC) are correlated with disease progression [5]. Amplification of MET and overexpression of MET has been reported in various tumors, including gastric and esophageal carcinomas [2,6,7]. Furthermore, MET amplification is a known acquired resistance mechanism to epidermal growth factor receptor (EGFR) inhibition in NSCLC [8–10]. Recent investigation has advanced in the approval of targeted therapy with MET tyrosine kinase inhibitors for metastatic NSCLC harboring a MET exon 14 skipping mutation [11–14]. Nevertheless, given the relative intolerance to the approved agents, continuing drug development efforts are ongoing to inhibit dysregulated MET signaling in various tumor types.

DO-2 ($C_{19}H_{12}D_1F_2N_7$) is the deuterated version of a first-generation highly selective MET kinase inhibitor (JNJ-38877605) [15]. This compound was found to be susceptible to metabolism by a species-specific isoform of Aldehyde Oxidase 1 (AOX-1) situated adjacent to the crucial hinge binding recognition site of MET [15–17]. However, the occurrence of presumed renal toxicity in the phase I trial of selective MET kinase inhibitors (JNJ-38877605 and SGX-532) hindered further clinical evaluation and development [15,17,18]. The observed serum creatinine increases with MET kinase inhibitors could be attributed to different mechanisms. Firstly, it has been reported that the observed renal adverse events resulted from the formation of insoluble metabolites, mediated by AOX-1, that precipitate in the kidney [15,17–19]. Notably, preclinical investigations indicated that only relatively high doses are being linked with the precipitation of an insoluble metabolite in kidneys, observed in rabbits [15]. Secondly, data suggests that serum creatinine increases are related to an on-target mechanistic cause linked to inhibition of organic cation transporter 2 (OCT-2) (unpublished data:

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data on record). Of note, JNJ-38877605 is a potent OCT-2 inhibitor and OCT-2 is involved in active secretion of creatinine [20]. Consequently, these two mechanisms potentially involved in renal adverse events with MET inhibitors, should be taken into account with further drug development efforts.

The plasma concentrations and area under the plasma concentration curve (AUC) in the phase I clinical trial of JNJ-38877605 were below the levels associated with antitumor activity in preclinical models [15]. Metabolism of JNJ-38877605 by AOX-1 into insoluble metabolites likely contributed to these sub-therapeutic exposures. In case of DO-2, deuteration induces a stronger bond between deuterium and the carbon atom, which is anticipated to reduce AOX-1 metabolism of DO-2 compared to the normal hydrogen carbon bond [21]. Preclinical investigations of DO-2 have shown that deuteration mitigates the metabolic liability to AOX-1, resulting in markedly reduced formation of M3. Therefore, in contrast to JNJ-38877605, the deuterated compound DO-2 is anticipated to yield exposures that correspond adequately with predicted efficacious plasma levels. Consequently, DO-2 emerges as a promising novel MET inhibitor currently undergoing clinical evaluation.

Pharmacokinetics are key to obtain pharmacologic understanding in dose finding trials, wherein plasma concentrations serve as main indicators to comprehend exposure-response and exposure-toxicity relationships. In addition, determination of plasma concentrations of novel anticancer agents enables the investigation of drug-drug interactions and combination therapies. Accurate determination of plasma concentrations of DO-2 and its metabolites DO-5 and M3 necessitates the development of a robust and fully validated ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) assay. Here, we describe the detailed set-up and validation process of the UPLC-MS/MS method to quantify DO-2 and its metabolites DO-5 and M3 in human plasma.

2. Experimental

2.1. Chemicals

The compounds DO-2, DO-5 and M3 were provided by Deuter-Oncology (Liege, Belgium). The internal standard, rolipram, was sourced from Sigma-Aldrich (Zwijndrecht, The Netherlands). Acetonitrile, methanol, and water were procured from Biosolve (Valkenswaard, The Netherlands), while dimethyl sulphoxide (DMSO) and ammonium formate were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Additionally, 2-propanol was acquired from Merck (Darmstadt, Germany) and formic acid from J.T. Baker BV (Deventer, The Netherlands). Blank human sodium citrated plasma was supplied by Sanquin Bloedbank Zuidwest (Rotterdam, The Netherlands), while blank lithium heparinized plasma and potassium EDTA plasma was donated by volunteers.

2.2. Preparation of stock solutions, calibration standards and quality control samples

Stock solutions of DO-2, DO-5 and M3 at a concentration of 1.00 mg/mL in DMSO were prepared in triplicate and stored at $T < -70$ °C. Stock solutions that met the accepted criteria of 5% difference from each other were allowed. A single stock solution of each compound was selected to prepare the working stock solution containing 40 μg/mL DO-2, 40 μg/mL DO-5 and 80 μg/mL M3, while another stock solution was used for preparing quality control samples. After aliquoting, these working stock solutions were stored at $T < -70$ °C and utilized for the preparation of calibration curve standards. The internal standard stock solution was prepared as 1 mg/mL rolipram in DMSO and stored at $T < -70$ °C. To create the internal standard working solution, 10 μL of 1 mg/mL rolipram was mixed with 90 μL DMSO. Hereafter, 10 μL of this solution was added to 100 mL acetonitrile, yielding an internal standard solution containing 10 ng/mL of rolipram. The internal standard working solution was stored at $T < 8$ °C.

Calibration standards were prepared in duplicate by diluting the working stock solution in acetonitrile/DMSO (1:1, v/v) and further diluted in human plasma to obtain final concentrations of 1000, 900, 500, 200, 50.0, 10.0, 2.50 and 1.00 ng/mL for DO-2 and DO-5, and 2000, 1800, 1000, 400, 100, 20.0, 5.00 and 2.00 ng/mL for M3.

The Lower Limit of Quantitation (LOQ) was set at 1.00 ng/mL for DO-2 and DO-5 and at 2.00 ng/mL for M3. Two distinct approaches were used to determine the LOQ. In the first approach this involved adding 10 μL of a working stock solution containing 20.0 ng/mL DO-2 and DO-5 and 40.0 ng/mL M3 to 190 μL of lithium heparinized plasma from ten different lots and nine different lots of sodium EDTA plasma. For the second approach, separate runs were conducted where a pool of LOQ samples was processed as quality control (QC) samples. Four pools of QC samples were prepared in human plasma with sodium citrate as coagulant at concentrations of 3.00 ng/mL (Low QC), 400 ng/mL (Middle QC), 800 ng/mL (High QC) and 8000 ng/mL (Diluted QC) for DO-2 and DO-5 and 6.00 ng/mL (Low QC), 800 ng/mL (Middle QC), 1600 ng/mL (High QC) and 16,000 ng/mL (Diluted QC). These samples were aliquoted and stored in cryo-vials at $T < -70$ °C.

2.3. Sample preparation

A plasma sample aliquot of 25 μL was mixed with 100 μL of the internal standard working solution (10 ng/mL rolipram in acetonitrile) in 1,5 mL safe-lock vials. Following vigorous mixing for 5 s, the vials were centrifuged for 10 min at 18,000 x *g* at ambient temperature. Subsequently, 50 μL of the clear supernatant was transported to a 96-well plate, whereafter an aliquot of 100 μL of water/formic acid/ammonium formate (100:0.1:0.02, v/v/v) was added. After mixing on a rocking platform for an additional 5 min, aliquots of 5 μL were injected onto the UPLC column.

2.4. Equipment

The UPLC-MS/MS system utilized in this study was procured from Waters Chromatography B.V. (Etten-Leur, The Netherlands) and comprised of a Waters Acquity FTN-1 Sample Manager and a Waters Acquity H-class Quaternary pump coupled to a Waters Xevo TQ-XS Detector. Data acquisition and processing was conducted using the MassLynx V4.2 SCN1007 software. Quantification was performed using TargetLynx as implemented in the software.

2.4.1. Chromatographic conditions

Analytes were divided using an Acquity UPLC ® HSS T3, 1.8 μm, 2.1 × 150 mm, column (Waters Chromatography B.V., Etten-Leur, The Netherlands). Column temperature was set at $T = 40$ °C. The mobile phase consisted of water/formic acid (100:0.1, v/v; mobile phase A) and acetonitrile/formic acid (100:0.1, v/v; mobile phase B), which were eluted at a flow-rate of 0.400 mL/min. A linear gradient was utilized, starting at T = 0 with 30% mobile phase B (70% mobile phase A). Initially, from T = 0 to T = 2.0 min, mobile phase B was increased from 30% to 50%. Afterwards, a further increase from 50% to 90% was effectuated from T = 2.0 to T = 2.1 min and maintained until T = 3.5 min. Subsequently, the conditions were restored to the initial situation between T = 3.5 and T = 3.6 min, with a regeneration time of 1.4 min. The total run time was 5 min. The needle wash solvent (wash through needle) consisted of a mixture of acetonitrile/methanol/water/2-propanol/formic acid (25:25:25:25:0.1, v/v/v/v/v).

2.4.2. Mass spectrometry

Tandem mass spectrometry was applied using positive ion electrospray ionization mode. Mass transitions with *m/z* values were optimized by directly infusing the respective analytes in acetonitrile/water/formic acid (40:60:0.1, v/v/v). The MS setup was manually adjusted to obtain optimal MS settings. The desolvation gas flow rate was set at 1000 L/h

and the cone gas flow was set at 150 L/h (nitrogen). The ionspray voltage was maintained at 4.50 kV, and the cone voltage was set at 40 V for DO-2, DO-5 and M3 and 45 V for rolipram. The source temperature was set at T = 150 °C, and the desolvation temperature was set at T = 650 °C. Dwell times were automatically optimized at 163 ms for rolipram and 38 ms for DO-2, DO-5 and M3. The inter channel delay was automatically managed. For quantitation in the Multiple Reaction Monitoring (MRM) mode, the following parameters were implemented: *m/z* 276 > 208 and collision energy 18 V for rolipram, *m/z* 379 > 180 and collision energy 43 V for DO-2, *m/z* 365 > 108 and collision energy 42 V for DO-5, and *m/z* 394 > 119 and collision energy 35 V for M3. The mass spectrometer monitored the column effluent from T = 1.2 to T = 4.2 min after the start of the MS method, while from T = 0 to T = 1.0 and T = 4.2 to T = 5.0 min the eluents was directed to waste.

2.4.3. Quantitation

Calibration curves were linear in the range of 1.00 – 1000 ng/mL for DO-2 and DO-5, and 2.00 – 2000 ng/mL for M3 in human plasma. The calibration curves were obtained by plotting peak area ratios of the analytes to the internal standard rolipram against their respective known concentrations. Linear regression analysis with a weight factor of 1/concentration^2 was applied to generate these calibration curves.

2.5. Method validation

The method validation was developed in accordance with the Food and Drug Administration (FDA) guideline for bioanalytical method validation [22,23]. This guideline describes the requirements for calibration curve, QCs, accuracy, precision, recovery and stability of the analyte in the matrix to ensure that the method is optimized for validation.

Blank human plasma samples obtained from plasma donors, which included ten different lots of lithium heparinized plasma and nine lots of sodium EDTA plasma, were analyzed to assess the potential presence of endogenous contamination. Furthermore, the calibration standards were processed and analyzed to ascertain if the detector's output exhibited a linear function of the concentration over the nominal range. Additionally, calculation of the accuracy and variation in each individual analytical run, as well as the within-run precision, the average accuracy and the between-run precision at each concentration level was calculated using SPSS version 28.01.0. To validate these parameters, five spiked human plasma pools (LOQ and QC samples) were assayed These assays were conducted independently and in quintuplicate, and the calibration curves were generated in duplicate. Besides, for LOQ validation, each individual lithium heparinized and sodium EDTA plasma samples were spiked at a concentration of 1.00 ng/mL for DO-2 and DO-5 and 2.00 ng/mL for M3 and quantitated in a separate run. In addition, the inference of potential co-administered drugs was tested in triplicate in a separate run at Low QC and High QC concentrations by diluting QC Diluted with plasma obtained from patient samples with known intake of co-medication. The following co-medication was investigated: dexamethasone, ketamine, morphine, oxycodone, fentanyl, paracetamol, celecoxib (Celebrex®), diclofenac, lyrica, neurontin, amitriptyline, haloperidol, pantoprazole, metoclopramide, ascal, bupivacaine, losartan, metoprolol, macrogol, nadroparin, teveten, valproic acid and zopliclone. Finally, the recovery and matrix effect of DO-2, DO-5 and M3 was determined at the Low QC and High QC concentration levels as earlier described [24].

2.6. Stability of DO-2, DO-5 and M3

The stability of DO-2, DO-5 and M3 in human plasma was tested at concentrations of QC Low and QC High in triplicate, both during four freeze-thaw cycles and at ambient temperature for a 5-day period. For the stability during freeze-thaw cycles, the samples were thawed at room temperature and refrozen again at *T < −70* °C for at least 18 h. The

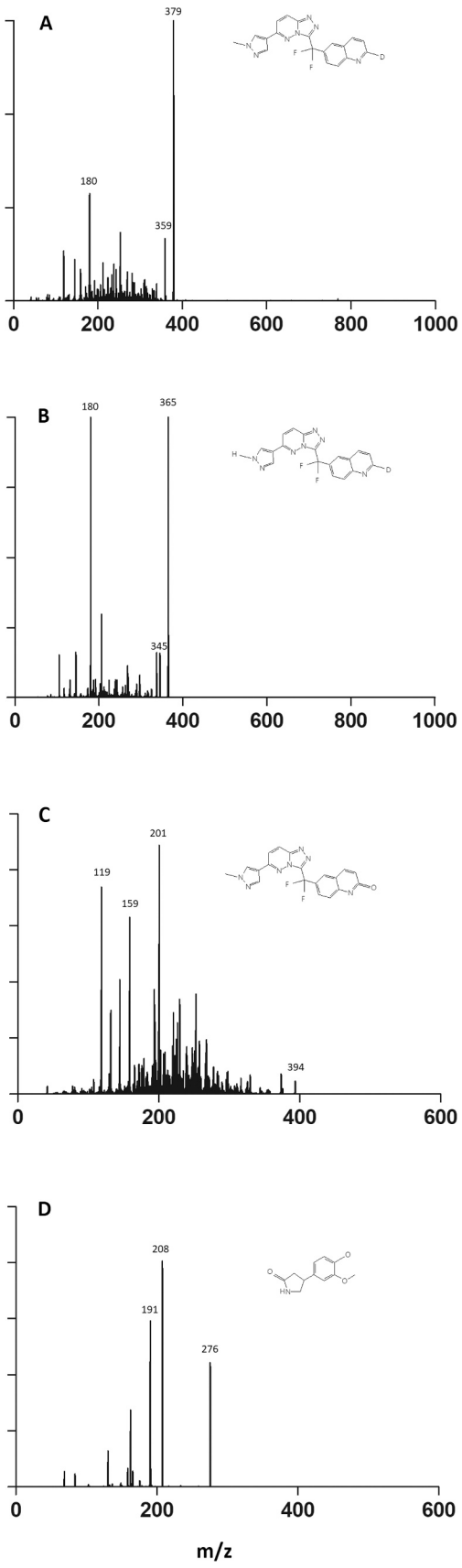

Fig. 1. Mass spectrum and chemical structure of DO-2, DO-5, M3 and the internal standard rolipram.

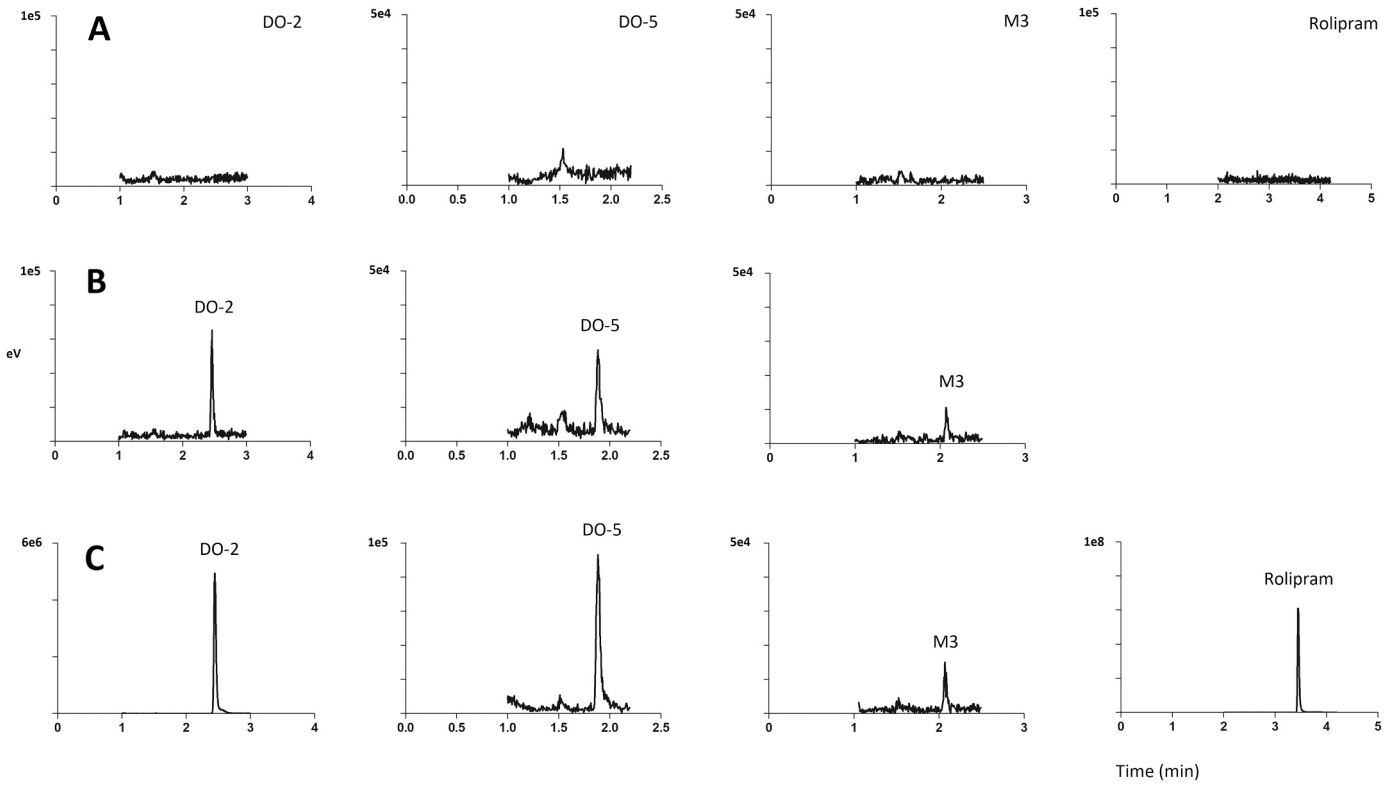

Fig. 2. Representative chromatograms of (A) double blank processed plasma samples, (B) plasma sample spiked at the concentration of the LOQ and (C) plasma sample collected 1.5 h after administration of 5 mg DO-2 on day 1 containing 85.6 ng/mL DO-2, 3.33 ng/mL DO-5, 3.09 ng/mL M3 and internal standard.

storage stability of processed samples in the autosampler was tested in triplicate at the concentration of QC Low and QC High. Long-term stability in human plasma was tested at the concentration of QC Low and QC High during storage at T < −70 °C and T < −20 °C.

2.7. Application of method to clinical samples

To illustrate the clinical utility of the validated bioanalytical method, blood samples were collected from a patient participating in an ongoing trial of DO-2 (NCT05752552). In the first cohort of this study, DO-2 was administered orally at a flat-fixed dose of 5 mg once daily. Furthermore, preliminary pharmacokinetic data was described for dose level 40 mg once daily.

3. Results and discussion

3.1. UPLC-MS/MS conditions

Product ion spectra of DO-2, DO-5 and M3 (Fig. 1) were detected by direct injection of these compounds into the mass spectrometer. Optimal sensitivity was obtained in the positive ion mode (electrospray positive). For quantitation, we used the product ions selected as the multiple reaction monitoring (MRM) ion transitions: *m/z* 179.9 for DO-2, *m/z* 180.1 for DO-5, *m/z* 119.0 for M3, and *m/z* 208.0 for the internal standard rolipram. Primary to secondary ion ratios of DO-2, DO-5 and M3 were used to show the quality of the observed peaks using ion transitions *m/z* 179.2/358.9 (ratio 11.0) for DO-2, *m/z* 180.1/345.1 (ratio 17.0) for DO-5 and *m/z* 119.0/201.0 (ratio 1.1) for M3. To separate potentially hydrophilic matrix components, eluting was applied at a flow rate of 0.400 mL/min with a linear gradient of mobile phase A (composed of water and formic acid, 100:0.1) and mobile phase B (composed of acetonitrile and formic acid, 100:0.1). The overall analysis time was 5 min were DO-2 elutes at 2.5 min and DO-5, M3 and rolipram at 1.7, 2.0 and 3.7 min respectively (Fig. 2). The use of the non-deuterated substance DO (DO-0) as internal standard was not suitable due to a high background signal. Therefore, rolipram was chosen as internal standard.

Table 1
Calculations of the between-run and within-run precisions and the average accuracy of the LLQ and QC samples[a].

Sample	Spiked (ng/mL)	GM (ng/mL)	ACC (%)	WRP (%)	BRP (%)	*n*[b]
DO-2						
LLQ	1.00	0.926	92.6	4.33	5.34	15 of 15
Low	3.00	3.02	100.7	3.01	5.06	15 of 15
Middle	400	380	95.0	1.57	2.68	15 of 15
High	800	744	93.0	2.03	0.81	15 of 15
Diluted	8000	7503	93.8	3.05	1.89	15 of 15
DO-5						
LLQ	1.00	0.956	95.6	5.36	6.67	15 of 15
Low	3.00	3.26	108.7	4.97	3.09	13 of 15
Middle	400	391	97.8	1.66	1.01	15 of 15
High	800	760	95.0	1.99	0.39	15 of 15
Diluted	8000	7905	98.8	2.97	1.96	15 of 15
M3						
LLQ	2.00	1.79	89.5	12.1	9.17	13 of 15
Low	6.00	5.86	97.7	4.49	4.65	15 of 15
Middle	800	770	96.3	1.40	2.97	15 of 15
High	1600	1522	95.1	2.25	1.80	15 of 15
Diluted	16,000	14,824	92.7	3.72	2.50	15 of 15

Abbreviations: GM, grand mean; ACC, average accuracy; WRP, within-run precision; BRP, between-run precision

[a] n = 5 in 3 separate runs

[b] Number of individual samples falling within acceptable range of accuracy of 85–115% (80–120% at LLQ)

3.2. Assay performance

The results of the method were linear over the concentration range of

Table 2
Matrix effect[a] and Recovery.

Condition	QC Low	QC High
DO-2		
Normalized matrix factor	0.81 ± 0.0247	0.88 ± 0.0109
Recovery	103 ± 2.09	102 ± 0.554
DO-5		
Normalized matrix factor	1.01 ± 0.0946	1.02 ± 0.0485
Recovery	119 ± 9.70	110 ± 3.54
M3		
Normalized matrix factor	0.84 ± 0.0136	0.89 ± 0.0118
Recovery	102 ± 2.78	99.6 ± 2.52

[a] Represents matrix factor of analyte / matrix factor of internal standard

Table 3
Stability.

Condition	(% to concentration at the initial time point)	
	QC Low	QC High
DO-2		
Ambient temp (5 days)	97.1	94.4
4 freeze/thaw cycles	103	100
Processed sample (T = 10 °C, 16 h)	95.6	96.0
T < −20 °C (217 days)	104	102
T < −70 °C (217 days)	94.7	91.4
DO-5		
Ambient temp (5 days)	96.4	95.8
4 freeze/thaw cycles	106	101
Processed sample (T = 10 °C, 16 h)	116	98.8
T < −20 °C (217 days)	99.5	102
T < −70 °C (217 days)	104	95.5
M3		
Ambient temp (5 days)	99.7	96.9
4 freeze/thaw cycles	104	102
Processed sample (T = 10 °C, 16 h)	106	97.9
T < −20 °C (217 days)	105	102
T < −70 °C (217 days)	86.3	88.5

1.00–1000 ng/mL with weighted linear regression of 1/concentration^2 in human plasma for DO-2 and DO-5, and 2.00–2000 ng/mL with weighted linear regression of 1/concentration^2 for M3. The LOQ was validated at a concentration of 1.00 ng/mL for DO-2 and DO-5, and 2.00 mg/mL for M3. For DO-2, accuracy was well within the acceptable range (80–120%) for ten independent lithium heparinized and EDTA plasma samples. For DO-5,the acceptable range of accuracy was met by eight of ten lithium heparinized and eight of nine EDTA plasma samples. For M3, nine of ten lithium heparinized and all EDTA plasma samples fell within the accepted accuracy range of 80–120%. The within-run and between-run precisions, as well as the accuracy at five predetermined concentrations, including LOQ, are summarized in Table 1. All values for DO-2, DO-5 and M3 align with the approval limits set by the FDA [22, 23]. Furthermore, the response for DO-2, DO-5 and M3 in a double blank processed sample injected directly after the highest calibration standard was ≤ 20% of the response at the LOQ. Therefore, no carry over was observed. The quantitation of DO-2, DO-5 and M3 remained unaffected by potentially co-administered drugs. No matrix effect was detected, and the recovery for all compounds was close to 100% at the concentrations of QC-Low and QC-High, across six different lots of human plasma (Table 2).

3.3. Stability

In human plasma, DO-2, DO-5 and M3 exhibited stability at ambient temperature for at least 5 days. Furthermore, these compounds were stable through four freeze-thaw cycles. Storage stability in the chilled (T = 10 °C) autosampler was tested for 16 h, revealing that DO-2, DO-5 and M3 remained stable as processed samples up to 16 h (Table 2). Long-term storage of plasma samples revealed stability after storage for at least 217 days at T < −70 °C and T < −20 °C (Table 3).

3.4. Clinical application

Representative plasma concentration versus time curves could be readily determined (Fig. 3). The measured concentrations indicate that the lower limit of quantitation of 1.00 ng/mL for DO-2 and DO-5 and 2.00 ng/mL for M3 is sufficient for monitoring drug-plasma levels in samples of patients treated with DO-2 at a flat-fixed dosage of 5 mg once daily. Ten blood samples were obtained within 24 h following the oral administration of DO-2 (i.e. before the subsequent dosage). Preliminary pharmacokinetic data was depicted for patients (n = 3) treated with DO-2 at a fixed-flat dosage of 40 mg once daily. This data revealed a maximum observed serum concentration (C_{max}) of 449 ± 50.8 ng/mL, 56.6 ± 26.6 ng/mL and 85.9 ± 16.1 ng/mL for DO-2, DO-5 and M3. The Area Under the Curve ($AUC_{0-24\,h}$) values were 1919 ± 290 ng*h/mL,

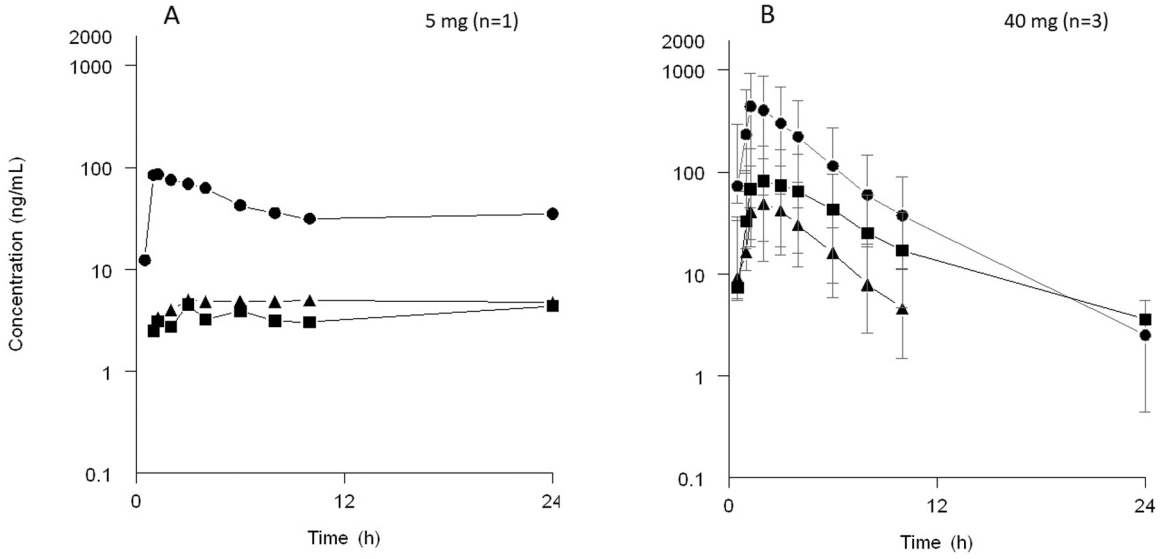

Fig. 3. Plasma concentration-time curve of DO-2 (-●-), DO-5 (-▲-) and M3 (-■-) in a patient following the administration of 5 mg (flat dose) (A) and average plasma concentration-time profile of DO-2 (-●-), DO-5 (-▲-) and M3 (-■-) in 3 patients following the administration of 40 mg DO-2 (flat dose) once daily in the first course. Pharmacokinetic parameters were calculated according to a non-compartimental analysis (Phoenix WinNonLin version 8.4; Pharsight, Mountain View, CA).

248 ± 104 ng*h/mL and 547 ± 135 ng*h/mL for DO2, DO-5 and M3.

4. Conclusion

In this study, we have presented an accurate, sensitive, reproducible and selective UPLC-MS/MS method, developed and validated for the quantification of DO-2, DO-5 and M3 in human plasma. The performance of the assay was successful for all three compounds, using rolipram as internal standard. The assay met all of the current requirements of bioanalytical method validation. Overall, this method will prove to be valuable for conducting pharmacokinetic analyses within the scope of clinical trials involving DO-2.

Ethics approval

The clinical trial of DO-2 is approved by the local ethics committee (Erasmus University Medical Center Rotterdam; MEC 2022-0603) and is registered at ClinicalTrials.gov (NCT05752552).

CRediT authorship contribution statement

Sikkema Barend J: Writing – original draft, Formal analysis. **Mathijssen Ron H.J:** Supervision, Funding acquisition, Conceptualization. **Robbrecht Debbie G.J:** Writing – review & editing, Conceptualization. **Perera Timothy P.S:** Writing – review & editing. **Koolen Stijn L.W:** Writing – review & editing, Funding acquisition, Conceptualization. **de Bruijn Peter:** Writing – original draft, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.RHJ Mathijssen reports a relationship with Boehringer Ingelheim Ltd that includes: funding grants. RHJ Mathijssen reports a relationship with Astellas Pharma Europe Ltd that includes: funding grants. RHJ Mathijssen reports a relationship with Bayer Corporation that includes: funding grants. RHJ Mathijssen reports a relationship with Cristal that includes: funding grants. RHJ Mathijssen reports a relationship with Novartis Pharmaceuticals Corporation that includes: funding grants. RHJ Mathijssen reports a relationship with PamGene International BV that includes: funding grants. RHJ Mathijssen reports a relationship with Pfizer Inc that includes: funding grants. RHJ Mathijssen reports a relationship with Sanofi that includes: funding grants. RHJ Mathijssen reports a relationship with Servier Switzerland SA that includes: funding grants. TPS Perera is the founder, director and a shareholder of DeuterOncology NV. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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