A sensitive LC-MS/MS method for relugolix quantification in human plasma and its application to a clinical study

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Overview

❖ **A sensitive LC-MS/MS method was successfully developed and validated:**

- Analyte: Relugolix (MVT-601)
- Analytical column: Phenomenex Gemini C18, 50 × 2.0 mm, 5-µm
- Calibration curve range: 0.05 – 50 ng/mL in 0.1-mL plasma
- Sample preparation: Liquid-liquid extraction with ethyl acetate
- Precision and accuracy:
  - Intra-day (n = 6): CV ≤ 3.6% (LLOQ ≤ 9.9%)
    RE -8.5 to 8.7% (LLOQ -4.0 to 11.0%
  - Inter-day (n = 18): CV ≤ 3.0% (LLOQ 8.3%)
    RE -7.8 to 8.0% (LLOQ 3.2%)
- Established stability: 6 F/T cycles; 23 hours at RT; 365 and 708 days frozen at -20 °C and -70 °C, respectively; Processed sample 141 hours at 2-8 °C

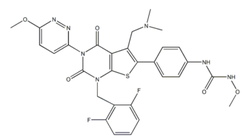
❖ **Application to a clinical study: The effect of moderate renal impairment on the relugolix PK**

- the $AUC_{0-\infty}$ and C_{max} of relugolix were increased by approximately 1.5-fold in subjects with moderate renal impairment compared to subjects with normal renal function.

Introduction

Relugolix, an oral active, nonpeptide gonadotropin-releasing hormone (GnRH) receptor antagonist that suppresses the production of testosterone, is approved in the United States, the European Union, and Canada for the treatment of patients with advanced prostate cancer. Previously published LC-MS/MS method for measuring relugolix in rat plasma has the LLOQ of 0.7 ng/mL [1]. We present a more sensitive LC-MS/MS method for relugolix quantification in human plasma that has the LLOQ of 0.05 ng/mL and its application to a clinical study on effect of moderate renal impairment on the pharmacokinetics of relugolix.

Relugolix



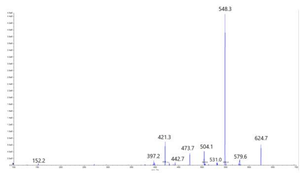
HPLC Parameters

Chromatography Settings							
Column type	Gemini C18, 2.0 × 50 mm, 5 µm, Phenomenex						
Column oven temperature	40°C						
Mobile phase composition	A: Water:Formic Acid at 100:0.1 (v:v) B: Methanol:Formic Acid at 100:0.1 (v:v)						
Program	Gradient						
Time (min)	0.0	1.0	1.4	1.5	1.9	2.0	2.7
%B	30	50	50	95	95	30	Stop
Flow Rate (mL/min)	0.5						
Autoinjector temperature	4°C						
Recommended wash solvent R0, R1 & R2:	Water: Formic Acid at 100:0.1 (v:v)						
Recommended wash solvent R3:	Acetonitrile:Methanol:Isopropanol:Water:Formic Acid at 30:30:30:10:0.2 (v:v:v:v:v)						
Analysis time	~3 min						
Injection volume	3 µL						
Retention time	RVT-601 = ~1.1 min [$^2H_6$]-RVT-601 (IS) = ~1.1 min						

MS/MS Parameters

Mass Spectrometer Settings (Recommended Values)	
Source Temperature (TEM):	600°C
Collision Gas (CAD):	6 psig N_2
Curtain Gas (CUR):	30 psig N_2
Ion Source Gas 1 (GS1):	60 psig N_2
Ion Source Gas 2 (GS2):	50 psig N_2
Ion Spray Voltage (IS):	5000 V
Entrance Potential (EP):	10 V
Scan duration:	1.5 min

Compound	Ionization Mode	Dwell Time (msec)	Declustering Potential (V)	Collision Energy (eV)	Collision Exit Potential (V)	Transition (m/z)
RVT-601	TIS+	150	70	35	15	624.2→548.2
[$^2H_6$]-RVT-601 (IS)	TIS+	150	70	35	15	630.2→548.2



Statistics of Back-Calculated Concentration of Calibration Standards

Analyte		Calibration standard concentration (ng/mL)								r^2
	Nominal	**0.0500**	**0.100**	**0.500**	**1.50**	**5.0**	**15.0**	**45.0**	**50.0**	
Relugolix	Back-cal. Mean (n=6)	0.0503	0.0982	0.517	1.54	5.22	14.8	44.0	47.5	≥ 0.9980
	S.D.	0.0019	0.00369	0.0122	0.0288	0.167	0.225	0.543	0.354	
	%CV	3.8	3.7	2.4	1.9	3.3	1.5	1.2	0.7	
	%RE	0.6	-1.8	3.4	2.7	4.4	-1.3	-2.2	-5.0	

Intra- and Inter-run Precision and Accuracy of QC Samples

Relugolix QC samples (ng/mL)						
Nominal		0.0500	0.150	2.00	20.0	40.0
Intra-run 1 (n=6)	Mean	0.0555	0.163	2.16	18.5	41.4
	S.D.	0.00176	0.00376	0.0480	0.214	0.250
	%CV	3.2	2.3	2.2	1.2	0.6
	%RE	11.0	8.7	8.0	-7.5	3.5
Intra-run 2 (n=6)	Mean	0.0512	0.162	2.07	18.3	40.5
	S.D.	0.00178	0.00548	0.0390	0.319	0.697
	%CV	3.5	3.4	1.9	1.7	1.7
	%RE	2.4	8.0	3.5	-8.5	1.3
Intra-run 3 (n=6)	Mean	0.0480	0.162	2.12	18.9	42.1
	S.D.	0.00475	0.00587	0.0420	0.569	0.959
	%CV	9.9	3.6	2.0	3.0	2.3
	%RE	-4.0	8.0	6.0	-5.5	5.3
Inter-run (n=18)	Mean	0.0516	0.162	2.12	18.6	41.3
	S.D.	0.00430	0.00484	0.0564	0.457	0.921
	%CV	8.3	3.0	2.7	2.5	2.2
	%RE	3.2	8.0	6.0	-7.0	3.3

Application: The Moderate Renal Impairment Study with Normal Healthy Comparators – Study Design and Results

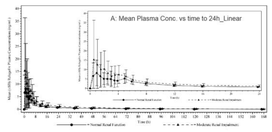
➢Study Design:

- An Open-Label, Single-Dose (40-mg relugolix) Study to Assess the Effect of Moderate Renal Impairment on the Pharmacokinetics of Relugolix
- Cohort 1: 12 participants with **normal renal function** classified by [Clcr] ≥ 90 mL/min C
- Cohort 2: 12 participants with **moderate renal impairment** classified by [eGFR] of 30-59 mL/min/1.73m^2
- Blood samples were collected on Day 1 at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, and 168 hours post dose for determining plasma relugolix concentrations.

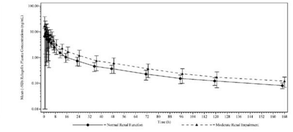
Cohort	Statistics	AUC_{0-t} (h*ng/mL)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	t_{max} (hr)	$C_{max(2)}$ (ng/mL)	$t_{max(2)}$ (hr)	$t_{1/2}$ (hr)	CL/F (L/h)	V_z/F (L)
Normal Renal Function (Cohort 1) (N=12)	n	12	12	12	12	10	10	12	12	12
	Mean (SD)	93.40 (37.14)	11.65 (6.825)	86.97 (35.59)	1.54 (1.29)	5.357 (2.509)	4.25 (1.32)	56.3 (14.3)	545 (388)	41900 (24600)
	CV% Mean	39.8	58.6	40.9	83.5	46.8	31.0	25.3	71.1	58.6
	Geo. Mean (CV%)	84.81 (54.0)	9.892 (67.9)	78.41 (56.7)		4.630 (71.7)				
	Median	96.65	9.040	91.17	1.25	5.495	5.00	49.9	414	36100
	Min, Max	23.62, 163.6	2.870, 23.60	20.30, 156.6	0.50, 5.00	1.050, 9.280	2.00, 6.00	42.3, 86.9	244, 1690	16000, 112000
Moderate Renal Impairment (Cohort 2) (N=12)	n	12	12	12	12	9	9	12	12	12
	Mean (SD)	144.6 (85.35)	20.92 (18.83)	135.3 (81.68)	1.25 (1.03)	6.439 (2.744)	3.67 (0.79)	56.3 (13.2)	385 (240)	29100 (13900)
	CV% Mean	59.0	90.0	60.4	82.7	42.6	21.6	23.4	62.5	47.9
	Geo. Mean (CV%)	123.2 (66.6)	14.57 (111.9)	114.8 (67.1)		5.940 (44.9)				
	Median	139.9	15.45	129.5	1.00	5.950	4.00	58.0	286	29200
	Min, Max	44.66, 343.2	4.270, 65.40	41.98, 331.9	0.50, 4.00	3.200, 11.70	2.50, 5.00	33.8, 76.9	117, 896	8160, 51500

Application: Effect of renal impairment on PK parameters

A: Mean Plasma Conc. vs time to 168h_Linear



B: Mean Plasma Conc. vs time to 168h_Semi-Log



Parameter	n	Mean (SD)	Geometric Mean[a]	Moderate Renal Impairment / Normal Renal Function Ratio Geometric Mean Ratio (SE)[a]	90% CI[a]
$AUC_{0-\infty}$ (ng*hr/mL)					
Moderate Renal Impairment (N=12)	12	144.6 (85.35)	123.2	1.4521 (0.3311)	(0.9812, 2.1491)
Normal Renal Function (N=12)	12	93.40 (37.14)	84.81		
AUC_{0-t} (ng*hr/mL)					
Moderate Renal Impairment (N=12)	12	135.3 (81.68)	114.8	1.4646 (0.3410)	(0.9816, 2.1854)
Normal Renal Function (N=12)	12	86.97 (35.59)	78.41		
C_{max} (ng/mL)					
Moderate Renal Impairment (N=12)	12	20.92 (18.83)	14.57	1.4712 (0.4641)	(0.8550, 2.5386)
Normal Renal Function (N=12)	12	11.65 (6.825)	9.892		

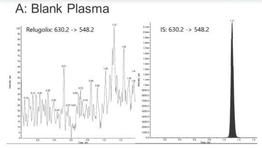
a: From an ANOVA model for the log-transformed parameter results with cohort as a fixed effect. Within cohort variances are not assumed to be equal. Satterthwaite's approximation is used to compute the degree of freedom for constructing the confidence intervals.

Sample Preparation Using Liquid-Liquid Extraction

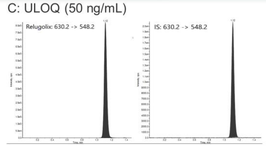
IS spiking	0.100-mL plasma sample + 25 µL IS working solution (60 ng/mL)
Extraction	Add 0.65-mL of ethyl acetate Mix thoroughly followed by centrifugation at 3000 rpm at RT for 5 mins
Transfer	Transfer 0.39-mL to a 96-well plate
Evaporation	Dry wells under nitrogen stream at 40 ºC
Reconstitution	Reconstitute with 0.15-mL of MeOH:Water:Formic acid (20:80:0.1, v:v:v)
Analysis	Inject 3-µL sample for LC-MS/MS analysis

Chromatograms of Plasma Blank, LLOQ, ULOQ , and a Clinical Sample

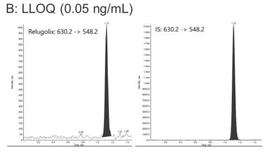
A: Blank Plasma



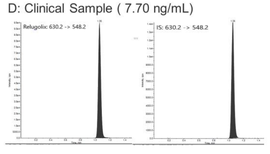
C: ULOQ (50 ng/mL)



B: LLOQ (0.05 ng/mL)



D: Clinical Sample (7.70 ng/mL)



Stability, Matrix Effect, Selectivity, and Extraction Recovery

Validation	Parameters	Results
Processed Sample Stability (n=6) [a]	141 Hours at 4°C	%RE: -7.5 to 10.7 %CV: 1.9 to 7.6
Benchtop Stability (Plasma) (n=6) [a]	23 Hours at Ambient Temperature	%RE:-3.3 to 1.3 %CV: 2.5 to 3.3
Freeze/Thaw Stability (n=6) [a]	6 Cycles at -20°C and -70°C	%RE: -4.3 to 10.0 (at -20°C) -0.8 to 12.0 (at -70°C) %CV: 3.0 to 4.0 (at -20°C) 3.3 to 6.4 (at -70°C)
Benchtop Stability (Whole Blood) (n=3) [a]	2 Hours at Ambient Temperature	%Diff [e]: 3.0 to 3.6
Long-term Storage Stability (n=6) [a]	365 Days at -20°C 708 Days at -70°C	%RE: 4.0 to -12.3 (at -20°C) 2.0 to 2.7 (at -70°C) %CV: 2.8 to 4.9 (at -20°C) 1.2 to 7.3 (at -70°C)
Dilution Integrity (n=5)	100 ng/mL diluted 20-fold	%RE: -1.2 %CV:3.7
Spike-in Selectivity (n=6) [b]	Spike-in 6 lots of human plasma	%RE: -1.8
Blank Selectivity (n=6)	6 lots of human plasma	No detectable relugolix and IS peaks
Hemolysis effect (n=5) [a]	2% hemolyzed K_2EDTA plasma	%RE: -9.3 to -12.3 %CV: 2.0 to 2.5
Lipidemic effect (n=5) [a]	Lipemic plasma	%RE: -10.7 to -13.0 %CV: 1.7 to 4.0
Recovery (n=5) [c]	Relugolix	Mean recovery: 78% %CV: 2.1
Matrix Effect (n=6) [a]	6 lots of human plasma	Mean IS-normalized MF: 0.978 and 0.994 %CV: 0.3 to 1.6

Note:
[a] Evaluated at two concentrations: LQC (0.150 ng/mL) and HQC(40.0 ng/mL)
[b] Evaluated at LLOQ (0.050 ng/mL)
[c] Evaluated at three concentrations: LQC (0.150 ng/mL), LMQC(2.00 ng/mL) and HQC (40.0 ng/mL)
[d] %Diff = ((mean of 2-hour peak area ratio - mean of 0-hour peak area ratio) / mean of 0-hour peak area ratio) x 100

Summary

- A sensitive LC-MS/MS method (LLOQ of 0.0500 ng/mL) was developed and validated for the quantitation of relugolix in human plasma in support of the clinical development of relugolix.
- This validated method has been successfully used in the measurements of relugolix in clinical samples including the presented clinical study: Effect of moderate renal impairment on PK parameters.
- The non-compartment PK analysis showed that the AUC0-∞ and Cmax of relugolix were increased by approximately 1.5-fold in subjects with moderate renal impairment compared to subjects with normal renal function.
- The clinical application demonstrated the present method is reliable for the quantification of relugolix in human plasma samples with sufficient sensitivity and assay robustness.

Reference:

– L. Xing, Y. Liu, H. Yao, T. Wang, F. Xie, S. Luo, P. Luo, and S. Tang. An Efficient UPLC-MS/MS Method Established to Detect Relugolix Concentration in Rat Plasma, *Front. in Pharmacol.*, 16 June 2022. https://doi.org/10.3389/fphar.2022.874973

Disclosures and Acknowledgement:

Authors declare no conflict of interest. This work was fully funded by Sumitomo Pharma Switzerland GmbH). Presented at: *2024 ASMS Annual Conference*.