

## Objective

## Introduction

Bicalutamide is an antiandrogen medication that is primarily used to treat prostate cancer. Bicalutamide is a racemate mixture with antiandrogenic activity by R-enantiomer while the S-enantiomer is essentially inactive. Thus it is important to qualify (R)-bicalutamide and (S)-bicalutamide for better understanding the therapeutic effect of bicalutamide. Here is presented a validated LC/MS/MS method with chiral separation of (R)-bicalutamide and (S)-bicalutamide in human plasma and prostate tissue.

## Method Summary

Matrix:

- Human plasma
- human prostate tissue homogenate

Method Calibration Range:

- 0.500-500 ng/mL for Human plasma
- 5.50-5500 ng/g for human prostate tissue homogenate

Sample Preparation:

human prostate tissue is homogenate after adding PBS:IPA (1;1) solvent at ratio of 10 mL of tissue homogenization reagent for every gram of tissue. (R)-bicalutamide and (S)-bicalutamide are extracted from human plasma or human prostate tissue homogenate by protein precipitation. Before the extraction, bicalutamide-d4 containing both R and S forms is added as internal standards. The upper layer is then transferred into a new plate and evaporated to dryness. The dried samples are reconstituted with water:IPA solution and injected into an LC/MS/MS system using an amylose-2 chiral column with acetonitrile:water:ammonium acetate mobile phases.

HPLC Condition:

- Lux Amylose-2 (150x4.6 mm, 3 μm)
- Gradient mobile phases: water:acetonitrile:ammonium acetate

Detection

- API4000 mass spectrometry

## Human Plasma Method Validation Data Calibrator Statistics

(S)-bicalutamide																	
Assay Date	Curve Number	STD 0.500 ng/mL	% Bias	STD 1.00 ng/mL	% Bias	STD 2.50 ng/mL	% Bias	STD 10.0 ng/mL	% Bias	STD 25.0 ng/mL	% Bias	STD 100 ng/mL	% Bias	STD 400 ng/mL	% Bias	STD 500 ng/mL	% Bias
01-Apr-20	1	0.503	0.6	0.952	-4.8	*1.81		10.3	3.0	26.1	-4.4	105	5.0	414	3.5	498	-0.4
02-Apr-20	3	0.521	4.2	0.951	-4.9	*0.655		9.81	-1.9	23.7	-5.2	101	1.0	392	-2.0	490	-2.0
30-Apr-20	11	0.517	-3.4	1.01	1.0	2.57	2.8	9.65	-3.5	24.6	-1.6	102	2.0	406	1.5	506	1.2
Mean		0.485	8.6	1.03	3.0	2.38	-4.8	10.7	7.0	25.5	2.0	93.2	-6.8	393	-1.8	500	0.0
Mean		0.503	3.0	1.05	5.0	2.33	-6.8	10.2	-2.1	26.1	-4.4	103	3.0	440	10.0	485	-3.0
S.D.		0.0331		0.0408		0.150		0.498		0.938		4.28		19.6		12.9	
%CV		6.6		4.1		6.3		4.9		3.7		4.3		4.8		2.6	
%Bias		0.6		0.0		-5.2		2.0		0.8		0.0		2.3		-1.6	
n		6		6		4		6		6		6		5		6	

(R)-bicalutamide																	
Assay Date	Curve Number	STD 0.500 ng/mL	% Bias	STD 1.50 ng/mL	% Bias	STD 380 ng/mL	% Bias	STD 500 ng/mL	% Bias	STD 1000 ng/mL	% Bias	STD 2500 ng/mL	% Bias	STD 5000 ng/mL	% Bias	STD 10000 ng/mL	% Bias
01-Apr-20	1	0.523	4.6	0.990	-1.0	*1.84		10.3	3.2	25.1	0.4	105	5.0	411	2.8	502	0.4
02-Apr-20	3	0.484	-3.2	0.981	-1.9	*0.662		9.68	-3.2	24.4	-2.4	99.9	-0.1	388	-3.0	494	-1.2
30-Apr-20	11	0.515	3.0	0.978	-2.2	2.55	2.0	10.3	3.0	25.8	3.2	94.9	-5.1	395	-1.3	503	0.6
Mean		0.482	-3.6	1.02	2.0	2.24	-10.4	10.3	3.0	26.0	4.0	96.9	-3.1	*487		463	-7.4
Mean		0.508	1.6	1.08	8.0	2.36	-5.6	9.88	-1.2	25.8	3.2	102	2.0	427	6.8	508	1.6
S.D.		0.499		1.01		2.42		10.0		25.3		99.6		405		496	
S.D.		0.0389		0.0383		0.150		0.463		0.912		3.60		15.1		16.9	
%CV		3.8		3.8		6.2		3.1		2.6		3.6		3.7		3.4	
%Bias		-0.2		1.0		-3.2		0.0		-1.2		1.3		-0.4		-0.8	
n		6		6		4		6		6		6		5		6	

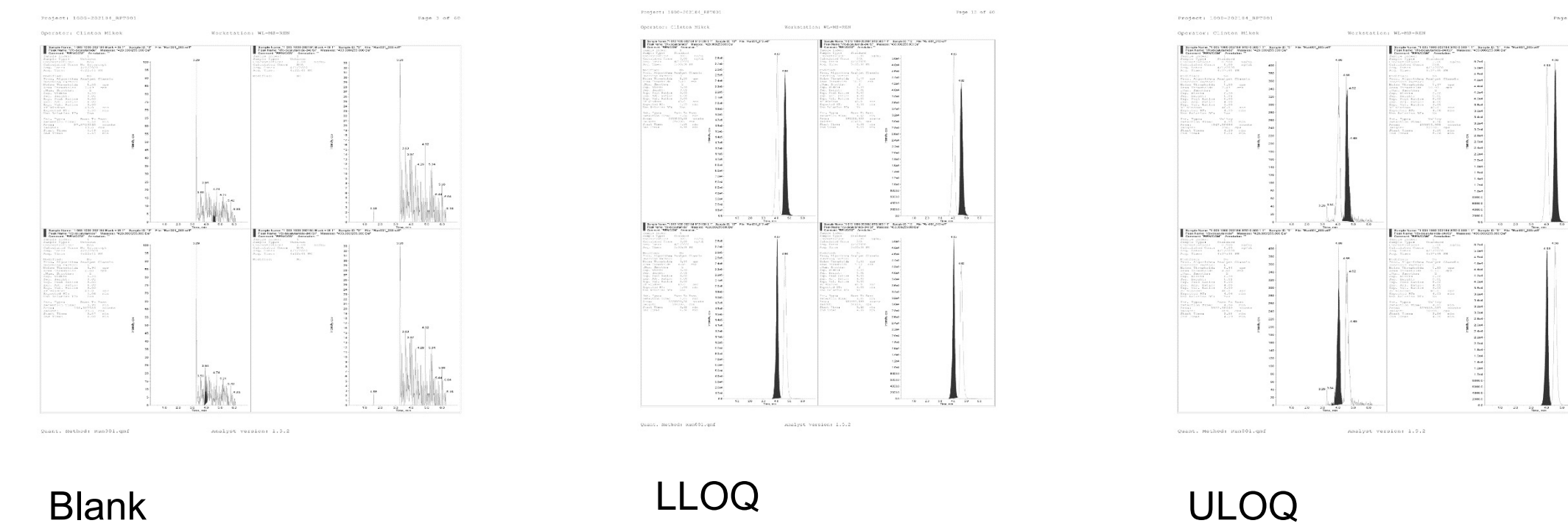
## QC Statistics

Run Date	Curve Number	(S)-bicalutamide						(R)-bicalutamide									
		VS 0.500 ng/mL	% Bias	VS 1.50 ng/mL	% Bias	VS 250 ng/mL	% Bias	VS 0.500 ng/mL	% Bias	VS 1.50 ng/mL	% Bias	VS 250 ng/mL	% Bias				
1-Apr-20	1	0.478	-4.4	1.41	-6.0	247	-1.2	398	4.7	0.496	-0.8	1.47	-2.0	246	-1.6	387	1.8
		0.489	-2.2	1.44	-4.0	242	-3.2	399	5.0	0.489	-2.2	1.37	-8.7	238	-4.8	390	2.6
		0.491	-1.8	1.42	-5.3	246	-1.6	377	-0.8	0.481	-3.8	1.43	-4.7	243	-2.8	380	0.0
		0.479	-4.2	1.39	-7.3	246	-1.6	389	2.4	0.473	-5.4	1.39	-7.3	247	-1.2	379	-0.3
		0.475	-5.0	1.41	-6.0	244	-2.4	384	1.1	0.456	-8.8	1.35	-10.0	241	-3.6	378	-0.5
		0.509	1.8	1.42	-5.3	243	-2.8	388	2.1	0.478	-4.4	1.41	-6.0	241	-3.6	382	0.5
2-Apr-20	3	0.519	3.8	1.41	-6.0	256	2.4	383	0.8	0.451	-9.8	1.41	-6.0	253	1.2	386	1.6
		0.552	10.4	1.48	-1.3	255	2.0	379	-0.3	0.524	4.8	1.44	-4.0	255	2.0	375	-1.3
		0.498	-0.4	1.48	-1.3	239	-4.4	390	2.6	0.516	3.2	1.43	-4.7	242	-3.2	392	3.2
		0.424	-15.2	1.44	-4.0	240	-4.0	394	3.7	0.455	-9.0	1.42	-5.3	236	-5.6	386	1.6
		0.507	1.4	1.41	-6.0	247	-1.2	380	0.0	0.557	11.4	1.47	-2.0	238	-4.8	379	-0.3
		0.472	-5.6	1.46	-2.7	243	-2.8	407	7.1	0.477	-4.6	1.44	-4.0	238	-4.8	403	6.1
30-Apr-20	11	0.472	-5.6	1.69	12.7	259	3.6	393	3.4	0.476	-4.8	1.67	11.3	250	0.0	392	3.2
		0.487	-2.6	1.64	9.3	274	9.6	446	17.4	0.511	2.2	1.62	8.0	273	9.2	445	17.1
		0.486	-2.8	1.55	3.3	252	0.8	427	12.4	0.481	-3.8	1.61	7.3	255	2.0	416	9.5
		0.481	-3.8	1.75	16.7	279	11.6	414	8.9	0.462	-7.6	1.67	11.3	286	14.4	425	11.8
		0.476	-4.8	1.65	10.0	264	5.6	411	8.2	0.468	-6.4	1.61	7.3	259	3.6	418	10.0
		0.475	-5.0	1.53	2.0	261	4.4	436	14.7	0.473	-5.4	1.66	10.7	250	0.0	426	12.1
Mean Concentration (ng/mL)		0.487		1.5		252		400		0.485		1.49		250		397	
Inter-run SD		0.0258		0.111		11.6		20.1		0.0273		11.3		13.0		20.6	
Inter-run %CV		5.3		7.4		4.6		5.0		5.6		7.5		5.2		5.2	
Inter-run %Bias		-2.6		0.0		0.8		5.3		-3.0		-0.7		0.0		4.5	
n		18		18		18		18		18		18		18		18	

## Validation Summary

Validation experiment	
Dilution Factor	10
Blank Selectivity	No interference with different lots of matrix, plus hemolysis, lipemic, and OTC spiked matrix
Spiked Selectivity	Meet criteria with with different lots of matrix, plus hemolysis, lipemic, and OTC spiked matrix
Extraction Efficiency	(S)-bicalutamide: ~120% (R)-bicalutamide: ~104%
Matrix Factor	No matrix effect with different lots of blanks
Long-term Stability	259 days at 20°C
Short-term Stability	24 hours at ambient condition
Freeze-thaw Stability	4 cycles at -20°C
Whole Blood Stability	72 hours at 2.8°C
Batch Repeatability	72 hours at 2.8°C
Processed Sample Stability	58 hours at 2.8°C
Stock and working Solution Stability	549 days at 2.8°C and 20 hours at ambient condition

## Typical Chromatograms



## Human Prostate Tissue Method Validation Data Calibrator Statistics

(S)-bicalutamide																	
Assay Date	Curve Number	STD 5.50 ng/g	% Bias	STD 11.0 ng/g	% Bias	STD 27.5 ng/g	% Bias	STD 110 ng/g	% Bias	STD 275 ng/g	% Bias	STD 1100 ng/g	% Bias	STD 4400 ng/g	% Bias	STD 5500 ng/g	% Bias
27-May-20	2	5.590	-2.0	11.300	2.7	27.4	-0.4	107.00	-2.7	276.0	1.1	1120	1.8	4490	2.0	5460	-0.7
28-May-20	4	5.590	1.6	10.800	-1.8	27.50	0.0	111.00	0.9	276.0	0.4	1080	-1.8	4420	0.5	5620	2.2
29-May-20	6	5.420	-1.5	11.20	1.8	27.20	-1.1	110.0	0.0	282.0	2.5	1080.0	-1.8	4410	0.2	5390	-2.0
Mean		5.660	2.9	10.70	-2.7	27.70	0.7	113.0	2.7	272.0	-1.1	1110.0	0.9	4490	2	5520	0.4
Mean		5.490	-0.2	10.60	-3.6	27.60	0.4	112.00	1.8	269.0	-2.2	1110	0.9	4350	-1.1	5440	-1.1
S.D.		0.1030		0.2060		0.252		2.070		4.670		18.60		55.3		90.0	
%CV		1.9		2.0		0.9		1.9		1.7		1.7		1.2		1.6	
%Bias		0.4		-0.9		-0.4		0.9		0.4		0.0		0.9		-0.5	
n		6		6		6		6		6		6		6		6	

(R)-bicalutamide																	
Assay Date	Curve Number	STD 5.230 ng/g	% Bias	STD 10.800 ng/g	% Bias	STD 27.8 ng/g	% Bias	STD 110.0 ng/g	% Bias	STD 286.0 ng/g	% Bias	STD 1130 ng/g	% Bias	STD 4530 ng/g	% Bias	STD 5330 ng/g	% Bias
27-May-20	2	5.230	-4.9	10.800	-1.8	27.8	1.1	110.0	0.0	286.0	4.0	1130	2.7	4530	3.0	5330	-3.1
28-May-20	4	5.480	-0.4	11.100	0.9	28.20	2.5	111.00	0.9	275.0	0.0	1080.0	-1.8	4400	0.0	5550	0.9
29-May-20	6	5.450	-0.9	11.100	0.9	26.80	-2.5	110.0	0.0	279.0	1.5	1070.0	-2.7	4450	1.1	5450	-0.9
Mean		5.620	2.2	*14.3		27.00	0.0	115.0	4.5	272.0	-1.1	1130.0	2.7	4540	3.2	5610	2.0
Mean		5.650	2.7	9.96	-9.5	27.00	-1.8	110.00	0.0	274.0	-0.4	1110	0.9	4260	-3.2	5350	-2.7
S.D.		0.2590		0.4910		0.645		2.000		5.							