A Simple, High-throughput, and Sensitive UPLC-MS/MS Assay to Quantify Abiraterone Acetate in Human Plasma

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INTRODUCTION

Abiraterone acetate (AA, **Figure 1**) is an orally administered medication to treat prostate cancer by inhibiting the 17α-hydroxylase and C17,20-lyase enzymatic activities of cytochrome P450 (herein referred to as CYP17). Both enzymes are required for androgen biosynthesis that is crucial in the progression from primary to metastatic prostate cancer. AA is also being used in clinical trials as a combination therapy for prostate cancer. Thus, we developed a simple, high-throughput, and sensitive assay to quantify AA in human plasma using ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS).

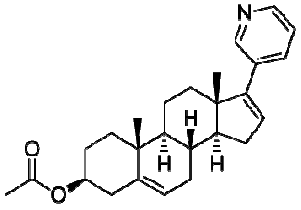

Figure 1: Chemical structure of abiraterone acetate

METHOD

Sample Preparation

Aliquots of 50.0 µL of human plasma containing AA and its internal standard (AA-d_4) were extracted using protein precipitation with acetonitrile (ACN). The extracts were analyzed using UPLC MS/MS.

LC-MS Conditions

UPLC: Agilent 1290 Infinity II LC System
Column: Advanced Materials Technology HALO ® 90 Å C18, 2.7 µm, 2.1 x 50 mm
Gradient profile: Refer to **Table 1**

METHOD (cont.)

Mobile phase A: 0.1% formic acid in H_2O
Mobile phase B: 0.1% formic acid in ACN
Needle wash: ACN/Methanol/IPA/Acetone (1:1:1:1. *v/v/v/v*)
Flow rate: 0.6 mL/min
Column temperature: 40 °C

Table 1: HPLC gradient profile

Time (min)	Events	Value
0.00	B conc.	40%
0.50	B conc.	40%
2.00	B conc.	75%
2.10	B conc.	90%
4.50	B conc.	90%
4.51	B conc.	40%
5.00	Stop	

SCIEX Triple Quad API 6500+ LC-MS/MS system was used in electrospray ionization (ESI) mode with positive ions to monitor AA and AA-d4 at ion transitions 392.5→332.2 and 396.5→336.2, respectively. A diversion valve was incorporated to reduce MS system contamination.

RESULTS & DISCUSSION

In order to develop a simple, fast and robustness assay, the stability, extraction efficiency and chromatographic separation were thoroughly optimized. The results of the stability study showed that AA is unstable in basic condition. However, AA has no stability issue under neutral and acidic conditions, even when subjected to high temperatures or strong illumination (**Figure 2**). The quantitative assay was successfully developed and quantified with a range of 0.100 to 100 ng/mL (**Figures 3 and 4, Tables 2 and 3**). The ranges of inter-assay accuracy (%Bias) for the lower limit of quantification (LLOQ) and other quality controls (QCs) were within 20% and within 15%, respectively.

RESULT & DISCUSSION (cont.)

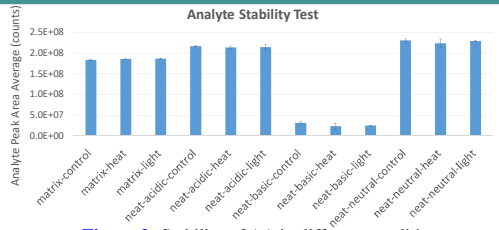

Figure 2: Stability of AA in different conditions

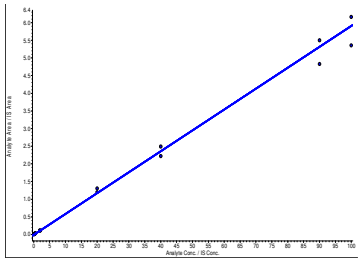

Figure 4: The typical calibration curve

Table 2: Calibration curve parameters

Batch ID	Slope	Intercept	Correlation Coefficient (R)
01	5.92E-02	6.52E-04	0.9970
02	6.11E-02	8.55E-04	0.9986
n	2	2	2
Mean	6.02E-02	7.54E-04	0.9978

Table 3: Precision and accuracy of calibration standards

	Concentration (ng/mL)							
Batch ID	0.100	0.200	0.500	2.00	20.0	40.0	90.0	100
01	0.106	0.200	0.560	1.98	22.0	42.0	93.0	104
	0.0930	0.195	0.510	1.83	20.3	37.5	81.6	90.5
02	0.0980	0.198	0.540	1.87	20.7	39.8	88.0	98.5
	0.0970	0.213	0.540	1.86	21.0	39.1	87.7	99.4
n	4	4	4	4	4	4	4	4
Mean	0.0985	0.202	0.538	1.89	21.0	39.6	87.6	98.1
SD	0.00545	0.00794	0.0206	0.0656	0.726	1.87	4.67	5.61
CV(%)	5.5	3.9	3.8	3.5	3.5	4.7	5.3	5.7
Accuracy(%)	98.5	101.0	107.6	94.5	105.0	99.0	97.3	98.1

RESULTS & DISCUSSION (cont.)

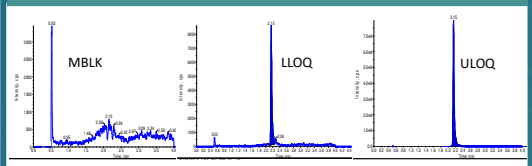

Figure 3: Typical chromatograms

Spiked QC sample precision and accuracy were demonstrated at *n*=12 at the LLOQ-QC (0.100 ng/mL), at low QC (0.300 ng/mL), medium QC (10.0 ng/mL), high QC (80.0 ng/mL). and dilution QC (1,000 ng/mL, dilution factor=25) over two runs (**Table 4**). The internal standard response variance (CV%) is as tight as 2.8% to 3.1% (**Table 5**).

Table 4: The inter-assay QC statistics

QC (ng/mL)	*n*	Mean	S.D.	%CV	Accuracy
0.100	12	0.103	0.00485	3.6	103.0
0.300	12	0.294	0.0317	2.0	98.0
10.0	12	9.63	0.7	1.1	96.3
80.0	12	76.8	1.17	1.0	96.0
1000	12	1030	49.8	4.8	103.0

Table 5: Internal standard response

Internal standard (IS):	Abiraterone Acetate-d4
IS response for accepted STDs and QCs (%CV):	2.8 to 3.1

CONCLUSIONS

- A sensitive and fast LC-MS/MS method for the quantification of abiraterone acetate was developed.
- A simple, high-throughput, and sensitive UPLC-MS/MS assay was developed and validated to quantify AA in human plasma.
- The method was quantified as linear, accurate, precise and reproducible. It can be used to determine the concentration of AA as low as 0.100 ng/mL using only 50.0 µL of sample.