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Characterization of the in Vitro Metabolic Profile of Abiraterone Acetate in cryopreserved Human Hepatocytes incubations by UPLC-Q Exactive Mass Spectrometry

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OBJECTIVE

This work demonstrated the application of technological combination of **UHPLC-Q-Exactive Orbitrap HRMS Analysis** with in vitro **human hepatocytes incubations** for the relatively complete analysis of non-polar (Phase I metabolites) and polar metabolites (phase II metabolites of **Abiraterone Acetate (AA).**

INTRODUCTION

Abiraterone Acetate is administered to patients with metastatic, castration-resistant prostate cancer and is metabolized into its active metabolite abiraterone and androgen receptor inhibitor D(4)-abiraterone (D4A). There were many studies covered on metabolites of abiraterone acetate (AA), however information on metabolites of abiraterone acetate in cryopreserved Human Hepatocytes was not reported. UHPLC in combination with high-resolution mass spectrometry (HRMS) provides an excellent analytical platform for the characterization of drug metabolites in drug discovery and development. Through this work, the above technology was successfully applied for the analysis of Phase I metabolites and phase II metabolites of Abiraterone acetate from human hepatocytes incubations. In total, three glucuronide metabolites, four sulfate metabolites, abiraterone and its other three major phase I metabolites were identified.

METHOD

1. 20 uM abiraterone was added into human hepatocytes incubations, samples at 0 hr and 4 hrs incubations were processed as cell pellets and medium fractions, Cells were lysed by mixing with 500 uL methanol;

2. Samples were rigorously vortexed and centrifuged at 15.000 rpm for 10 mins. The supernatant portion was transferred into vials and subjected for UPLC-HRMS analysis in order to identify abiraterone metabolites;

3. Structural elucidation of metabolites was performed using data-dependent MS/MS acquisition and collision-induced dissociation (CID) fragmentation; Raw data were processed by Xcalibur and Metamass site software;

METHOD (cont.)

LC-MS Conditions:

Chromatographic Conditions:
HPLC: Shimadzu LC-30AD
Columns: ACE C18-PFP column (150×2.1 mm, 3 µm)
Column temperature: 40 ºC

Mobile phase A: 0.1% formic acid , 1.0 mM Ammonium acetate in water/ACN (80/20);
Mobile phase B: 0.1% formic acid , 1.0 mM Ammonium acetate in MeOH/ACN (80/20);
Needle wash:
Acetonitrile/Methanol/IPA/Water (1:1:1:1, v/v/v/v)
Flow rate: 0.3 mL/min

HPLC gradient profile:

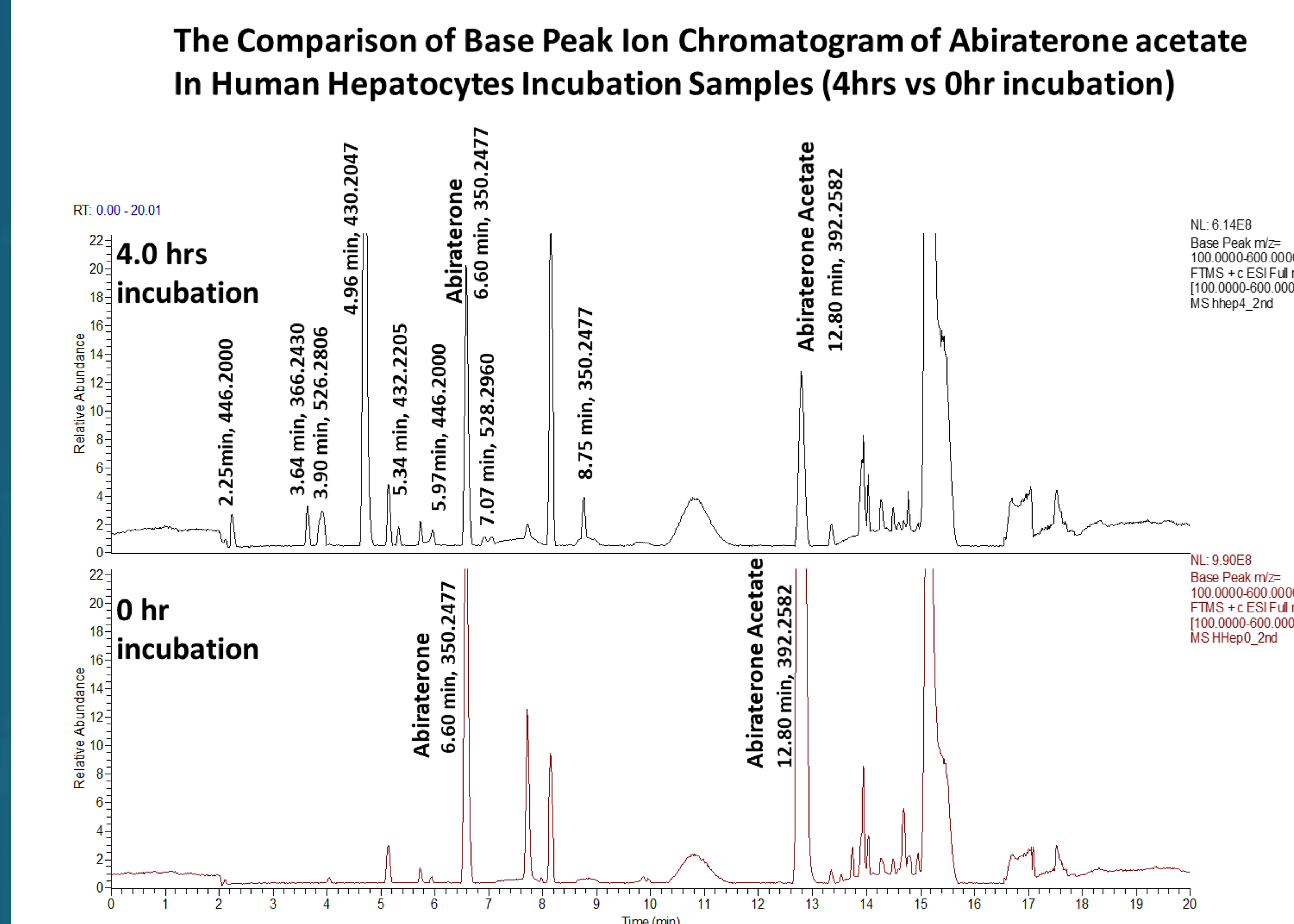
No	Time	Flow [µl/min]	%B	%C	Curve
1	0.000	Run			
2	0.000	300.000	10.0	0.0	5
3	6.000	300.000	55.0	0.0	5
4	9.500	300.000	55.0	0.0	5
5	10.000	300.000	75.0	0.0	5
6	12.000	300.000	75.0	0.0	5
7	12.100	300.000	10.0	0.0	5
8	17.000	300.000	10.0	0.0	5
9	*New Row*				
10	30.000	Stop Run			

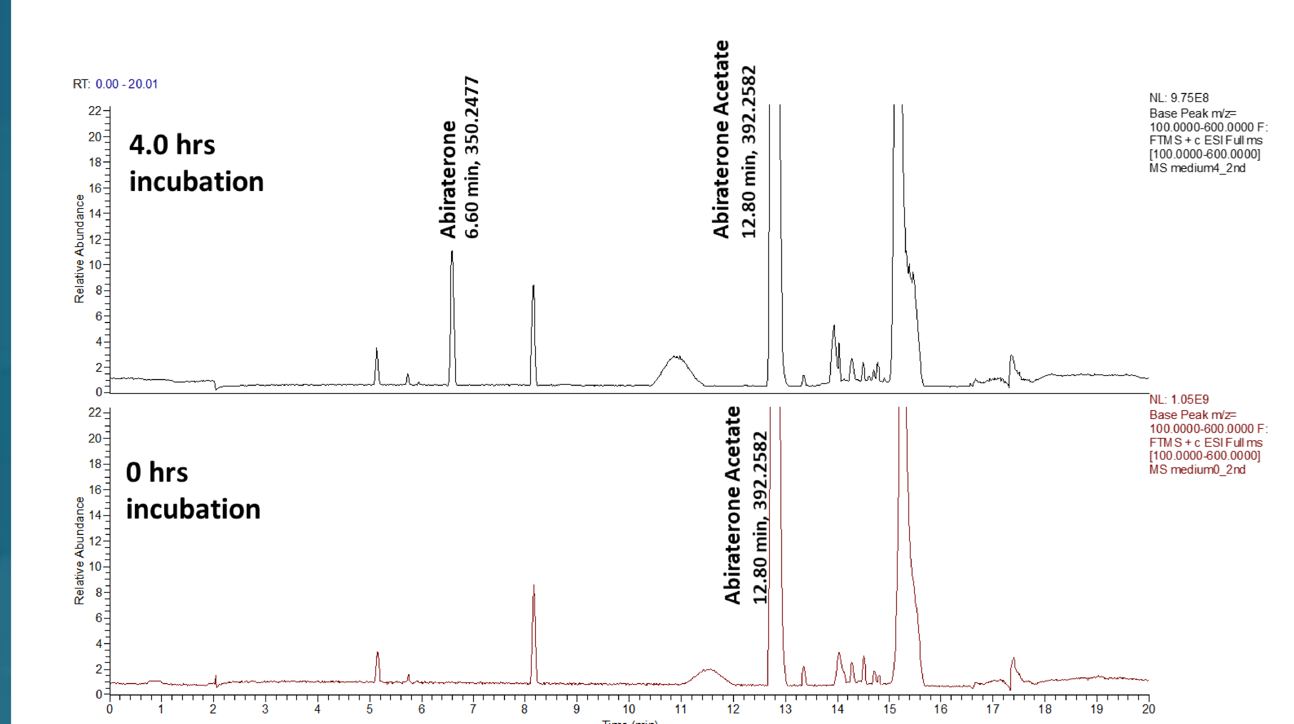
QE mass spectrometry settings:

Tandem mass spectrometry was performed with a Q Exactive Orbitrap MS (Thermo Fisher, Waltham, MA, USA) using a heated electrospray ionization source for the ionization of the target compounds in the positive ion mode. The operating parameters were as follows: spray voltage, 3.00 kV; sheath gas pressure, 30 psi; auxiliary gas pressure, 10 arb; capillary temperature, 320°C; auxiliary gas heater temperature, 350°C; scan modes, full MS (resolution 70,000) and ddMS2 [resolution 17,500, with stepped collision energy (15, 30, and 45 eV)]; and scan range, m/z 300–600. All data were acquired using the Xcalibur 3.1 software (Thermo Scientific).

RESULT

Representative chromatogram of hepatocyte cell lysate and cell culture media samples



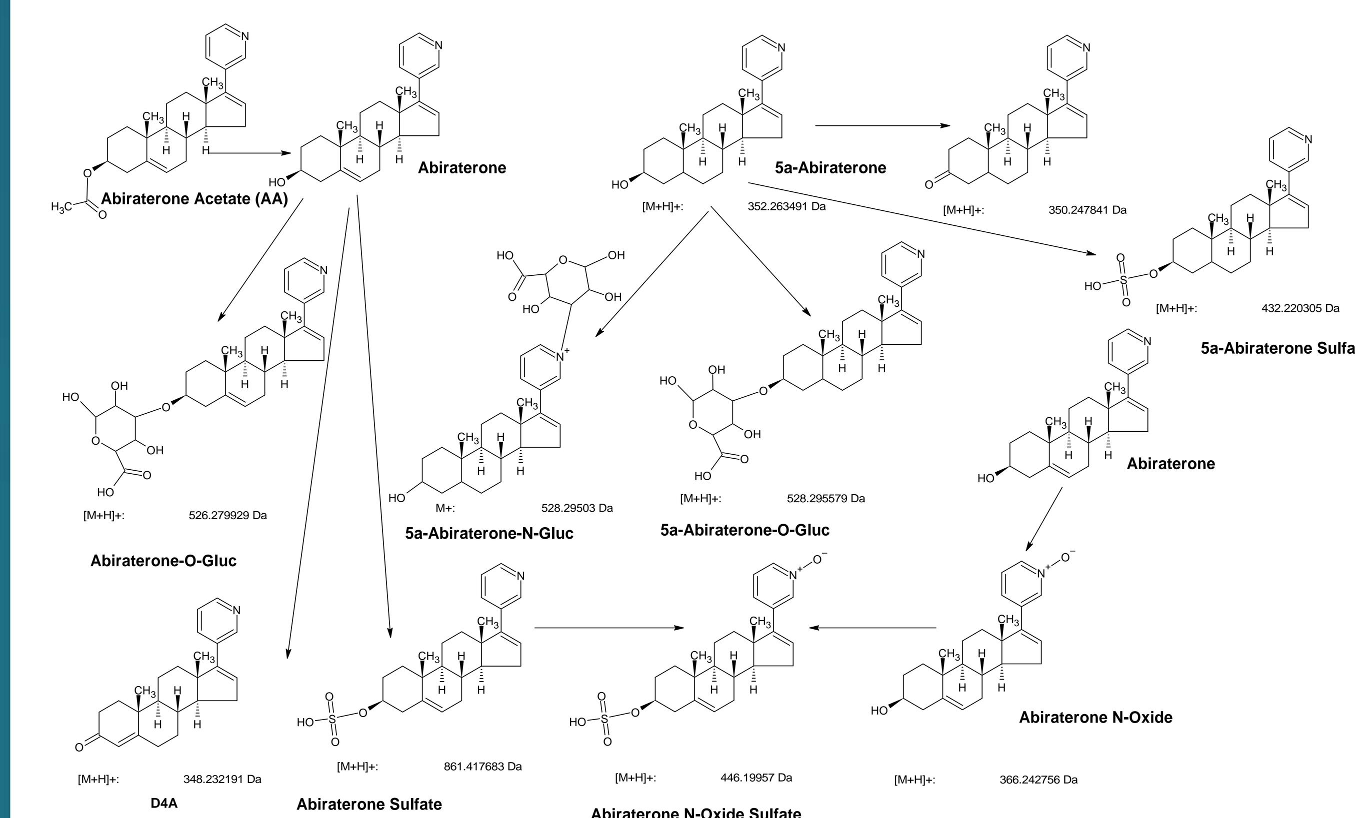


Detected Metabolites of Abiraterone Acetate in Human Hepatocyte incubations

Detected Metabolites of Abiraterone and Proposed Types				
Detected HRMS ion (m/z)	Predicted HRMS (m/z)	Retention time (mins)	MS2 fragments ions (m/z)	Proposed metabolite Types
446.2000	446.1995	2.25	446.1997; 348.2322; 366.2429; 333.2093;320.2370	**N-O+Sulfate**
366.2430	366.2427	3.64	366.2430; 350.2113; 318.1861	**Monoxidation (or N-oxide)**
526.2805	526.2799	3.91	526.2804; 332.2374; 350.2480	**Glucuronide**
430.2049	430.2046	4.69	430.2050; 350.2479;332.2373;317.2137	**Sulfate**
432.2205	432.2203	5.34	432.2206;352.2635; 334.2530	**Sulfate**
446.2000	446.1995	5.97	446.1998; 348.2324; 366.2430; 334.2155; 316.2062	**+O+Sulfate**
348.2322	348.2322	7.28	348.2322; 333.2089; 318.1851;157.0887	**D4A**
350.2477	350.2478	6.60	350.2479;334.2167;320.2009; 157.0886	**Abiraterone**
528.2960	528.2950	6.91	334.2529; 352.2635; 334.2529	**+2H_N-Glucuronide**
528.2960	528.2955	7.07	334.2529; 352.2635;334.2529	**+2H_O-Glucuronide**
350.2477	350.2478	8.75	350.2478; 335.2240; 320.2007; 170.0962; 157.0886	**5α-Abiraterone**
392.2582	392.2589	12.80	392.2583, 332.2371, 302.1901	**Abiraterone Acetate**

RESULT(cont.)

The Proposed Human Hepatocyte Metabolic Pathways of Abiraterone Acetate



CONCLUSIONS

1. Three glucuronide metabolites (with MS2 spectra of 176 Da neutral loss) and four sulfate metabolites (MS2 spectra with 80 or 98 Da loss) were identified; Abiraterone, its more potent form D4A, one isomeric metabolite and one major oxidation phase I metabolite were also identified;
2. All these metabolites were characterized all together for the first time from human hepatocytes incubation samples by UHPLC with HRMS;
3. These metabolites were not detected from cell medium samples which indicated the metabolites of AA were not excreted from human hepatocyte cells to medium during the incubation process.
4. This work demonstrated the technology platform is very useful in the analysis of non-polar (Phase I metabolites) and polar metabolites (phase II metabolites) of AA. In addition, the chromatographic conditions had to be optimized for an improved and adequate separation of isomeric metabolites.

REFERENCES

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