

## Analysis Report

<b>Project Name</b>	Untargeted Metabolomics Analysis
<b>Sample Description</b>	Bladder Cancer
<b>Sample Quantity</b>	12
<b>Order Number</b>	CPMO10071901
<b>Client</b>	
<b>Project Date</b>	2020-04
<b>Remark</b>	

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## ***Part 1 Samples***

12 bladder cancer cell samples, for untargeted metabolomics analysis in the collected samples by UPLC-MS.

## ***Part 2 Materials and methods***

### ***2.1 Instruments and reagents***

Ultimate 3000LC combined with Q Exactive MS (Thermo)  
Temp functional Centrifugation (Eppendorf)  
Acquity UPLC HSS T3 (100×2.1mm×1.8 µm)  
Acetonitrile (Merck)  
Methanol (Merck)  
Formic acid (CNW)  
Ultrapure water (Merck)

### ***2.2 Sample preparation***

- 1) Samples were thawed and add with 800 µL of 80% methanol followed by sonication for 30 min, 4 °C;
- 2) Then all samples were kept at -20 °C for 1 h;
- 3) After that, samples were vortexed for 30 s, and centrifuged at 12000 rpm and 4 °C for 15 mins.
- 4) Finally, 200 µL of supernatant was transferred to vial for LC-MS analysis.

## ***Part 3 LC-MS***

Separation is performed by Ultimate 3000LC combined with Q Exactive MS (Thermo) and screened with ESI-MS (targeted MS/MS mode). The LC system is comprised of an Acquity UPLC

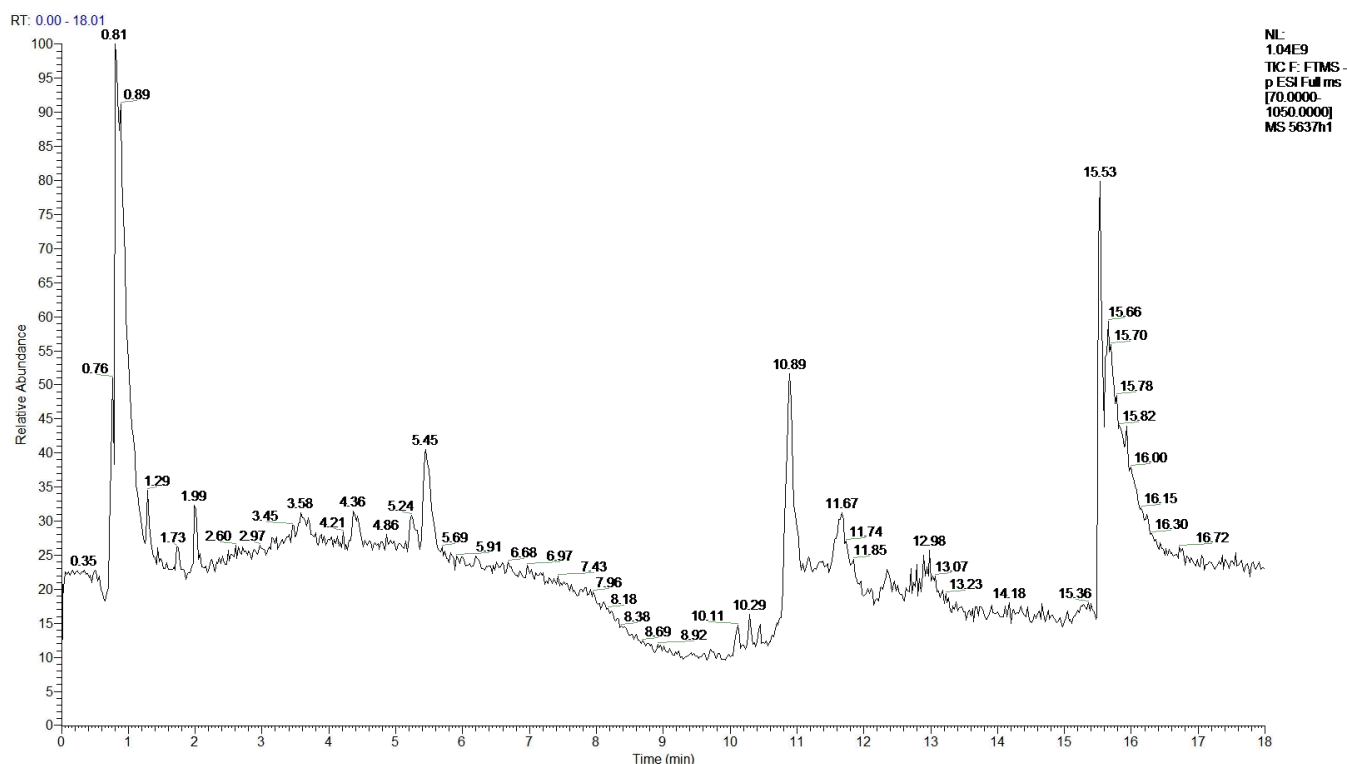
HSS T3 (100×2.1mm×1.8 μm) with Ultimate 3000LC. The mobile phase is composed of solvent A (0.1% formic acid-water) and solvent B (acetonitrile) with a gradient elution (0-1.5 min, 95-70% A; 1.5-9.5 min, 70-5% A; 9.5-14.5 min, 5% A; 14.5-14.6 min, 5-95% A; 14.6-18.0 min, 95% A). The flow rate of the mobile phase is 0.3 mL·min<sup>-1</sup>. The column temperature is maintained at 40°C, and the sample manager temperature is set at 4°C.

Mass spectrometry parameters in ESI<sup>+</sup> and ESI<sup>-</sup> mode are listed as follows:

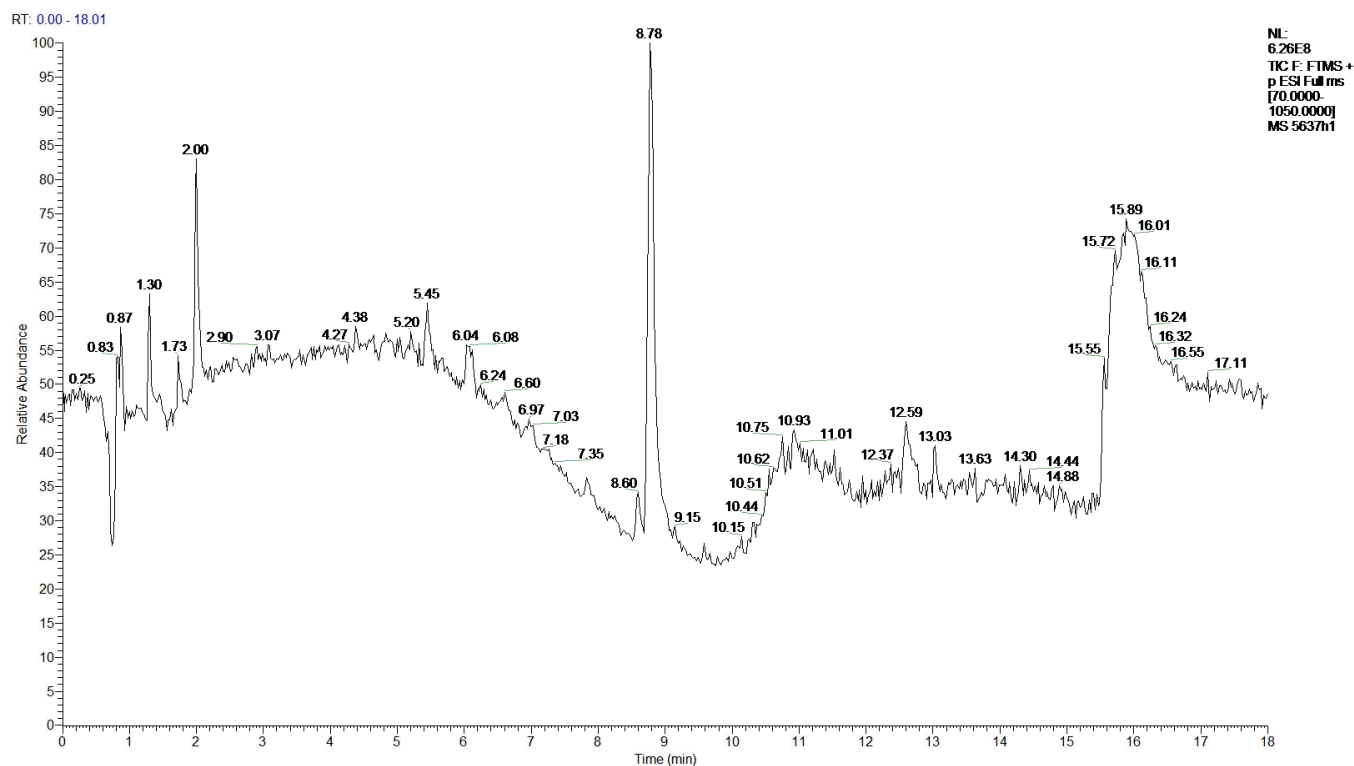
ESI<sup>+</sup>: Heater Temp 300 °C; Sheath Gas Flow rate, 45arb; Aux Gas Flow Rate, 15arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.0KV; Capillary Temp, 350 °C; S-Lens RF Level, 30%.

ESI<sup>-</sup>: Heater Temp 300 °C, Sheath Gas Flow rate, 45arb; Aux Gas Flow Rate, 15arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.2KV; Capillary Temp, 350 °C; S-Lens RF Level, 60%.

## Part 4 Analytical Results



**Figure.1** TIC obtained from cell in ESI<sup>-</sup> mode.



**Figure.2** TIC obtained from cell in ESI+ mode.

The results are shown in the excel sheet.