Glucocorticoid therapy is widely used for cancer chemotherapy, immunosuppression, and anti-inflammatory purposes. Prednisone is one of the most common drugs used for the therapy, however there is a general activation of catabolic metabolism. Chemotherapy, immunosuppression, and anti-inflammatory therapy, however, increases in urine production and extracellular elimination. 

In addition to the classical in vivo metabolism experiments, metabolic studies play an important role in assessing drug efficacy, drug safety, and mechanisms of action. Along with the quantification of the drug and its metabolites, profiling of other physiological fluid constituents can determine other metabolic processes that are affected by drug therapy. 

Metabolic profiling of physiological fluids with plasma or urine is complicated by the number of endogenous components (samples complex) and the number of analyses needed to gather meaningful statistical data.

Ultra performance liquid chromatography (UPLC) is characterized by the high chromatographic efficiency and high speed analyses achieved by the use of sub 2 μm particles. In addition to high efficiency and high speed chromatographic analysis, time of flight mass spectrometry is utilized to obtain high sensitivity full scan data that can be used for identification purposes. Accurate mass measurements generated from this high sensitivity full scan data that can be used for identification purposes. Accurate mass measurements generated from this can be used for identification purposes.

Pretreatment of urine was collected from an adult male during treatment with Prednisone (0 mg/day) for two days, with subsequent dosing of the drug. Aliquots were frozen at -40°C until analysis. Samples were thawed and diluted with 1.600 μL of 10X matrix, and then diluted with 10 volumes of water prior to analysis.

Liquid Chromatography/Mass Spectrometry

Waters ACQUITY UPLC

Mobile Phase

D 0% Acetonitrile/Formic Acid

Column: ACQUITY BEH C18 2.1x100 mm 1.7 μm dp

Flow Rate: 400 μL/min

Column Temperature: 50°C

LockSpray using Leucine Enkephalin as a reference mass

Collision Energy = 4 eV

Capillary: 3.5 kV

ESI Positive or Negative Mode

MSE Experiments

Collision Energy: 18 eV

W Mode resolution (fwhm) = 15,000 @ m/z 481.2443

LockSpray using Leucine Enkephalin as a reference mass

ESI Positive or Negative Mode

LockSpray using Leucine Enkephalin as a reference mass: W Mode resolution fwhm = 15,000 m/z range 15,000 m/z to 356 m/z

W Mode resolution (fwhm) = 15,000 @ m/z 481.2443, PCA Scores Plot showing group separation, and PCA Loadings Plot of components leading to group separation. Source File labeled replicates to Day 0 (mock-dose) through Day 6.

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References


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