Extraction of Urinary Hormone Metabolites using Supported Liquid Extraction prior to HPLC-MS/MS Analysis

Kristin Jones¹, Tifanie Vansachi¹, Kristyn Astern¹, Victor Vandel³, Bruce Kemp⁶, Dan Menasco¹ & Elena Gairloch¹

¹Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte North Carolina 28269, USA
²Physicians Lab, 4950 Communications Ave, Boca Raton, FL 33431, USA

Introduction

Estrogen, androgen, and glucocorticoid metabolism can be used to assess overall hormonal balance during hormone therapy. Urine is the recommended testing matrix for quantification of primary estrogen levels as well as secondary estrogen metabolites when monitoring overall hormone balance, therapy, and detoxification. Non-invasive collection allows for sampling over a 24-hour period, providing insight into a patient’s circadian rhythm. Since many samples are generated for a single patient in any one day, a fast and robust testing protocol is needed for extraction and analysis during clinical testing. Here, we demonstrate a rapid and reliable sample preparation method using Support Liquid Extraction (SLE+) to extract a suite of 30 hormone analytes from a hydrolyzed urine matrix. Single injection analysis by LC-MS/MS shows that matrix effects are eliminated by the SLE+ protocol and that analytic recovery and sensitivity have excellent clinical utility.

Experimental Reagents

Standards were obtained from Steraloids (Newport, RI) and Cerilliant (Round Rock, TX). 6-glucuronidase, ammonium acetate, formic acid, ammonium hydroxide, sodium bicarbonate, dansyl chloride, ammonium formate Sigma-Aldrich (St. Louis, MO). Dichloromethane, ethyl acetate and LC-MS grade solvents were obtained from Fisher-Scientific (Waltham, MA). Synthetic Negative Urine was obtained from Golden West Biologicals (Temecula, CA).

Sample Preparation

Hydrolysis: A Tecan Freedom EVO liquid handling system was used to transfer 500 µL of urine, 60 µL internal standard solution, and 600 µL of 100 mM NaHCO3 to a 20 µL droplet of LC-MS Grade H2O. Samples were then reconstituted with 100 µL of 1 mg/mL dansyl chloride and incubated at 60 °C for 5 minutes in order to selectively dansylate estrogen metabolites. The reaction was quenched by the addition of 25 µL of LC-MS Grade H2O.

Mass Spectrometry Parameters

Sample Application: Hydrolyzed urine (380 µL) was applied to each well of the SLE+ plate. After a 5 minute wait, positive pressure (1 bar) was applied to ensure complete absorption of each sample.

Conclusions

Sample pre-treatment: Various pH control strategies were evaluated, pH adjustment post-hydrolysis was eliminated from the protocol since all analytes were neutral in the hydrolysis buffer (pH 4.8). Sample pre-treatment: Various pH control strategies were evaluated, pH adjustment post-hydrolysis was eliminated from the protocol since all analytes were neutral in the hydrolysis buffer (pH 4.8).