Development of a rapid LC-MS/MS method for hair cortisol determination to assess the HPA axis

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Introduction

Hair cortisol is becoming an increasingly utilized biomarker for the assessment of the hypothalamic-pituitary-adrenal axis (HPA). To date, the majority of research has been in the areas of stress and psychoendocrinology,[1-4] but it has also been used for clinical evaluations, such as for Cushing syndrome,[5, 6] cardiovascular disease,[7, 8] childhood obesity,[9] and more recently asthma.[10] The determination of hair cortisol concentrations has predominantly been performed using enzyme-linked immunosorbent assays (ELISA). While a number of groups have developed LC-MS methods for the detection of cortisol in hair, the methods were developed for various indications and include the detection of other steroid compounds leading to long running times.[11-16] In order to improve turn-around-times, decrease costs compared to longer LC-MS/MS methods, and improve sensitivity and specificity over ELISA for our research needs, we are developing a rapid UHPLC-MS/MS method for the detection of cortisol in hair in a 2-minute run time.

Sample Collection and Preparation

Hair samples were collected from the vertex posterior of the head or pooled hair was collected for preparation of hair matrix for standard curves. Hair was washed with isopropanol and dried prior to extraction. Cortisol was extracted with methanol from a 10 mg hair sample, which is minced with scissors and incubated overnight as per our lab's established ELISA protocol.[8] The supernatant was either treated with solid phase extraction (SPE) using a C_{18} cartridge,[12] and evaporated under N_2 prior to reconstitution with 50:50 methanol:distilled deionized water with 0.1% formic acid, or evaporated under N_2, reconstituted, and then filtered with a 4 mm, 0.2 µm filter prior to analysis. Standard curves were prepared by spiking hair matrix with cortisol standard to give final concentrations of 0, 5, 20, 50, 100, 200, and 500 ng/g of hair (filtered) and

0, 0.05, 0.5, 5, 25, and 50 ng/g hair (SPE) based on a 10 mg hair sample. A deuterated cortisol-D_4 internal standard was added to all samples.

UHPLC Method

Samples were analyzed using an Agilent 1290 Infinity HPLC equipped with a Phenomenex Kinetex XB-C18, 50 x 2.1 mm, 1.7 µm column. A 10 µL sample was injected onto the column. The mobile phase gradient was 50%-90%-50% mobile phase B (100% methanol with 0.1% formic acid). Mobile phase A was 100% distilled deionized water with 0.1% formic acid. The flow rate was maintained at 600 µL/min.

Mass Spectrometer Method

The mass spectrometer used for analysis was the AB SCIEX QTRAP® 5500 LC/MS/MS in negative electrospray ionization (ESI) mode. Multi-reaction monitoring (MRM) was used for analyte detection, and the cortisol transition was 407.2 to 335.1 m/z and cortisol-D_4 transition was 411.2 to 339.1 m/z. Additionally, potential interference from the presence of cortisone (405.1 to 329.1 m/z) and prednisone (403.1 to 327.1 m/z.) were assessed.

Results

With this method, results can be obtained for cortisol in a run time of 2.0 minutes. The retention time for cortisol and cortisol-D_4 was 0.82 ± 0.01 min. The area count of the zero standard was subtracted from all other standard's area counts to correct the standard curve for endogenous cortisol levels in the pooled hair matrix used to prepare the calibration curve. The standard curve weighted $1/x$ was linear with a correlation coefficient (r) of ≥0.998. The limit of detection (LOD) was calculated using the slope and standard deviation (SD) of the y-intercept for each calibration curve (Equation 1).

Equation 1. $LOD = 3.3(SD) \div slope$

Not surprisingly, the calibration curve samples pretreated with SPE produced the lowest LOD at 0.12 ng/g. Because filtering the samples is a much faster process than performing SPE, future method development plans include assessing alternate filters for sample pretreatment to

determine if the LOD can be improved from the current value of 5.4 ng/g. Furthermore, the sample intra-day coefficient of variation was 2.5%.

Hair also contains a measureable amount of cortisone; therefore, we determined if the cortisone peak would interfere with the cortisol peak using our method (Figure. 1). Cortisone was found to have minimal contribution to the cortisol peak with a retention time of 0.74 min. Because this method is principally intended for the determination of hair cortisol levels in patients with asthma for our current research, we also chose to test for interference by a common corticosteroid medication used to treat patients with asthma, prednisone. The retention time for prednisone was 0.72 min and did not interfere with the cortisol peak (Figure. 1). Additionally, because patients with asthma use inhaled corticosteroids, further testing will be performed to determine if any interference occurs with the inhaled corticosteroids available on the market, or their metabolites as necessary. Ultimately, the goal is to develop the method to measure the hair cortisol and inhaled corticosteroid level in the same method, if possible.

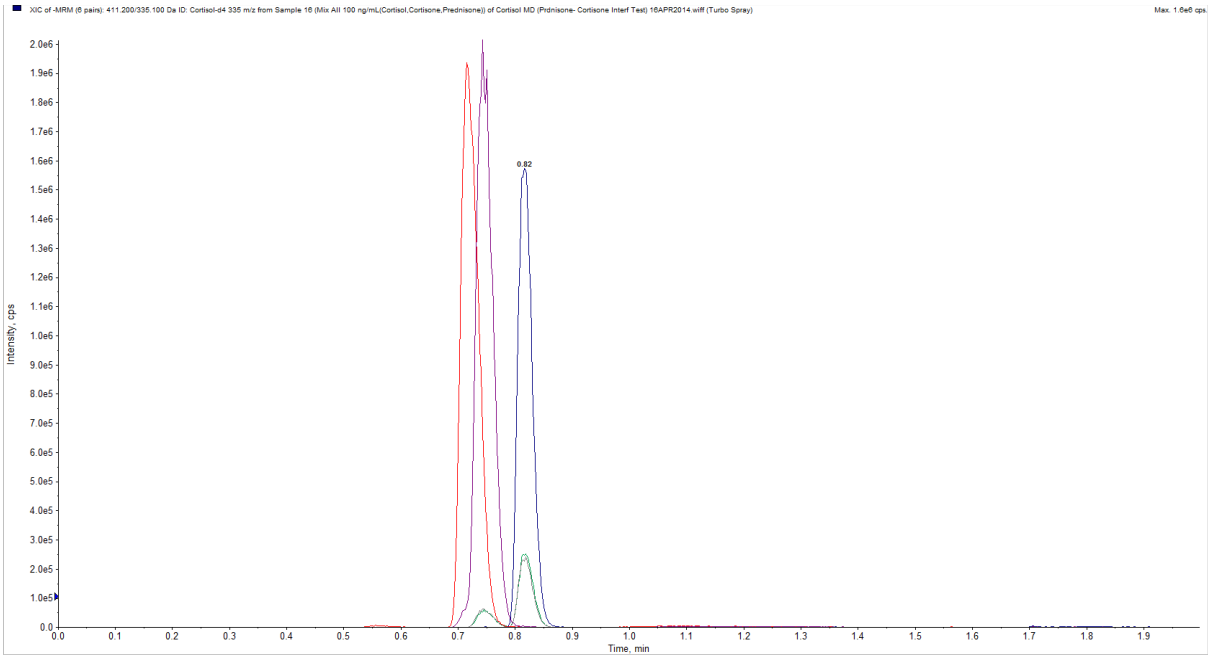

Figure. 1. UHPLC chromatogram for prednisone, cortisone and cortisol. Starting from the left, prednisone (red) is shown with a retention time of 0.72 min., followed by cortisone (purple) at 0.74 min, and cortisol (green) and cortisol-D_4 (blue) are at 0.82 min. It can be seen that while the prednisone and cortisone peaks are close to the cortisol peak, interference is nil and negligible, respectively.

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