Sensitive High Pressure Liquid Chromatography Mass Spectrometry (LCMSMS) for Detection and Potential Correlation of Steroids in Human Sweat and Blood

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**Purpose**

A survey of the literature has shown new trends focusing on sweat as an alternative, less invasive biofluid for analysis of target molecules including health biomarkers, indicators of illness such as cystic fibrosis, drugs of abuse and other stimulants. Novel wearable sensors are being developed at University of California, Berkeley, University of Cincinnati, the Air Force Research Laboratories and other research centers to measure the level of specific analytes in sweat to monitor various health conditions. The techniques used included gas chromatography-mass spectrometry (GC/MS), Liquid chromatography mass spectrometry (LCMS) and specific approaches such as enzyme linked immunosorbent assay (ELISA). The purpose of this study is to develop a sensitive diagnostic analytical method for determination of steroid hormones in non-invasively obtained samples of human sweat. The concentration of steroid hormones in circulation is extremely low and is dependent on both biological and environmental factors such as age, hormonal cycle in females, stress, pregnancy, etc. The ultimate goal is to establish a potential correlation between sweat and blood levels for diagnostic applications. As for the measurement of the level of steroid hormones, it is still primarily performed on blood, urine, or saliva samples using specific ELISA or radioimmunoassay kits or chromatographic techniques such as electrophoresis or LCMS. Sweat testing offers noninvasive sampling with little risk and discomfort to the patient.

This poster describes the potential use of sweat as an alternative matrix to blood for determination of steroid levels from individuals. A single and sensitive LC/MS/MS method was developed for measurement of the following hormones: Cortisol, Testosterone, β-Estradiol, Estrone and Progesterone in sweat using electrospray positive mode. Dehydro-epiandrosterone sulfate (DHEAS) was analyzed in a separate method using electrospray negative mode. The techniques were applied to sweat samples collected from male and female volunteers.

**Methods**

Iontophoresis was performed for 5 minutes on the ventral side of the forearms of male and female volunteers. Sweat samples were then collected using custom skin patches. Each patch was made of a commercially available filter paper affixed to a surgical film lined with a plastic adhesive layer to prevent evaporation. The patch was placed directly above the location of iontophoresis on the ventral side of the forearms. The patches were removed after 30 minutes and the absorbent filter was immediately placed into a custom extraction insert for microcentrifuge tubes. The filter was centrifuged for 10 minutes at 13,000 rpm. The extracted volume was weighed and was transferred to vials for storage at -20°C until analysis. The extracted volume collected varied among volunteers and ranged between 10-100 μL.

The filtrate was transferred to 200 μL inserts in HPLC vials for analysis. Cortisol, Testosterone, and Progesterone were analyzed in a single injection using electrospray positive mode (ESI+). The chromatographic separation of the components was achieved on a C18 Accucore column and gradient program with a mobile phase consisting of 0.6% acetic acid in water and methanol. DHEAS was analyzed in electrospray negative mode (ESI-) using the same gradient program. It was analyzed separately and combined with the other analytes.

**Results**

The methods were developed using commercially available simulated sweat and successfully applied to human sweat samples collected from patches worn by 11 volunteers (7 men and 4 women). The results of analysis of progesterone of three samples collected at distinct stages during the menstrual cycle of one female volunteer showed cycle-dependent increases and decreases as would be expected in blood. DHEAS and Cortisol were detected in the same eight volunteers. The values ranged from 0.06 - 14.96 ng/patch with an average 3.93 ng for DHEAS, and 0.9 - 3.7 ng/patch with an average 1.4 ng for cortisol.

**Conclusion**

The analytical methods consist of simple, rapid and easy-to-perform processes without requiring extensive sample treatment in comparison to other biofluids, especially blood. Due to increased interest in minimally- and non-invasive techniques for use in clinical laboratory, this study, produced a promising alternative to biomarker analysis for the determination of hormones in sweat. As the cost and availability of more sensitive mass spectrometers allows for their increased use and the technique of sweat collection become more efficient, this platform for assessment may have extensive applications.