

Using LC-MS/MS to Determine Salivary Steroid Reference Intervals in a European Older Adult Population

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A number of steroids, including glucocorticoids and sex hormones, have been associated with neurodegenerative and cardiovascular conditions common in aging populations. The application of liquid chromatography tandem mass spectrometry (LC-MS/MS) steroid analysis offers an opportunity to conduct simultaneous multiplex steroid analysis within a given sample. In this paper, we describe the application of an LC-MS/MS steroid analysis method for the assessment of reference ranges of steroids in human saliva samples (200 µL) collected from older adults (age 50 years and above) enrolled in a European cohort investigating the risk for Alzheimer's dementia. Saliva samples were prepared using supported liquid extraction (SLE) along with a calibration curve and analysed using a Waters I-Class UPLC (Ultra Performance Liquid Chromatography) and a Sciex QTrap 6500+ mass spectrometer. Mass spectrometry parameters of steroids were optimised for each steroid and a method for the chromatographic separation of 19 steroids was developed. Lower limits of quantitation (LLOQs), linearity and other method criteria were assessed. In total, data from 125 participants (500 samples) were analysed and assessed for reference ranges (64 male, 61 female). A total of 19 steroids were detected in saliva within the range of the method. There were clear diurnal patterns in most of the steroid hormones detected. Sex differences were observed for androstenedione (A4), testosterone (T), cortisone (E) and aldosterone (Aldo). In the first sample of the day, dehydroepiandrosterone (DHEA) was significantly higher in healthy volunteers compared to those with Alzheimer's disease biomarkers. This LC-MS/MS method is suitable for the analysis of 19 steroids in saliva in adults.

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